IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU, P53 & P63 IN UROTHELIAL BLADDER CARCINOMA AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES

Dissertation submitted in

partial fulfillment of the requirements for the degree of

M.D. PATHOLOGY

BRANCH- III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI- 600003

THE TAMILNADU DR M.G.R.MEDICAL UNIVERSITY

CHENNAI

MAY 2018

CERTIFICATE

This is to certify that this Dissertation entitled **"IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU, P53 & P63 IN UROTHELIAL BLADDER CARCINOMA AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES"** is the bonafide original work of **DR.K.KOKILA**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in May 2018.

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DECLARATION

I, **Dr.K.KOKILA,** solemnly declare that the dissertation entitled **"IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU, P53 & P63 IN UROTHELIAL BLADDER CARCINOMA AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES"** is the bonafide work done by me at the Institute Of Pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.Geetha Devadas,** M.D., DCP, Professor of Pathology and **Dr.R.NARMADHA,** M.D., Assistant professor of Pathology, Institute Of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

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CERTIFICATE OF APPROVAL

To
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Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"IMMUNOHISTOCHEMICAL EXPRESSION OF HER-2, P53,P63 IN UROTHELIAL BLADDER CARCINOMA AND ITS CORRELATION WITH CLINICO - PATHOLOGICAL VARIABLES " NO.24092016 .**

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC ,Ch-3	Deputy Chairperson
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9. Tmt.ArnoldSaulina, MA., MSW.,	Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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ABBREVIATIONS

TURBT	:	Transuretheral resection of bladder tumor
BCG	:	Bacille Calmette Guerin
WHO	:	World Health Organisation
ISUP	:	International Society of Urological Pathology
CK	:	Cytokeratin
ASCO	:	American Society of Clinical Oncology
MI	:	Muscle invasive
NMI	:	Non muscle invasive
H&E	:	Hematoxylin & Eosin
HER 2	:	Human Epidermal growth factor receptor 2
MMP	:	Matrix metallo proteinase
TIMP	:	Tissue inhibitor of metallo proteinase
HRP	:	Horse radish peroxidase
SCC	:	Squamous cell carcinoma
FISH	:	Fluorescence in situ hybridization
TCC	:	Transitional cell carcinoma
DAB	:	Diamino benzidine
NAT2	:	N-Acetyl transferase 2

CONTENTS

Introduction

INTRODUCTION

Urothelial carcinoma of the bladder is a major cause of morbidity and mortality throughout the world. Urothelial carcinoma comprises approximately 90% of all primary bladder tumors and is typically seen in patients over 50 years of age but is occasionally seen in younger adults and is rare in children[1].

It is approximately three times as common in men as in women. The pathogenesis of these tumors depend on a combination of genetic and environmental factors[2,3,4]. Among the latter, chemical factors are thought to be of great importance[4].

Bladder tumors are common in industrial areas (especially in those associated with petrochemicals [5,6], and their incidence is increased with exposure to cigarette smoke and arylamines [7,8].Other environmental factors include aniline dyes (particularly benzidine and beta naphthylamine [9,10], auramines, phenacetin and cyclophosphamide)[11,12,13].

It has been postulated that urinary tryptophan metabolites may be the endogenous counterparts of the carcinogenic dyes[14]. Schistosoma haematobium is also thought to be pathogenetically related to urothelial and squamous cell carcinoma of the bladder[12,15]. In contrast to renal cell carcinoma, patients with carcinoma of the bladder only exceptionally have systemic symptoms or paraneoplastic syndromes or present with metastatic disease. The majority of patients present with hematuria, although dysuria is also not infrequent, tending to be more common in patients with high grade tumors.

Urothelial carcinoma is conventionally divided into two types,the papillary and nonpapillary(flat-sessile) types. This distinction reflects two genetic pathways believed to account for the majority of urothelial carcinomas [16,17]. Noninvasive papillary carcinomas account for approximately 75% of newly diagnosed primary urothelial tumors of the bladder, 10% to 20% of these patients will however subsequently have an invasive tumor. Looked at from another angle, approximately 20% of patients with invasive bladder cancer have had prior noninvasive papillary lesions[18].Carcinoma in situ is most often seen with high grade papillary urothelial carcinoma; de novo carcinoma in situ accounts for only 1% to 3% of newly diagnosed urothelial carcinomas[19].

The treatment of urothelial carcinoma is largely based on histological grade and stage [20,21,22]. For noninvasive papillary tumors the primary therapy is transuretheral resection of the bladder tumor (TURBT). For low grade tumors a single intravesical treatment with mitomycin, doxorubicin, or epirubicin may be added; for high grade tumors intravesical Bacille Calmette Guerin(BCG) is the treatment of choice. Urothelial carcinoma in situ is managed as for high grade urothelial carcinoma.For the most part T1 tumors are treated the same way, and in general a repeated transuretheral resection is performed to exclude the presence of muscularis propria invasion[21]. Increasingly, patients with T1 tumors are being treated by cystectomy particularly if features are indicative of a high risk of progression to muscle invasive disease[22,23]. Once muscularis propria invasion is documented,cystectomy becomes the treatment of choice[22]. The role of

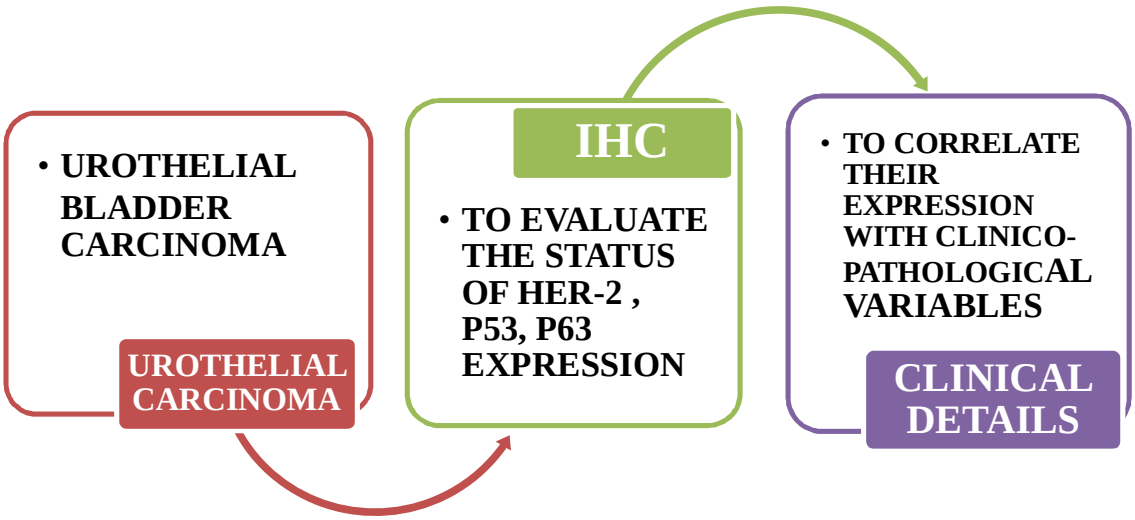
neoadjuvant and adjuvant chemotherapy for metastatic disease is under evaluation.

In this study, we evaluated the status of HER-2 NEU, P53, P63 expression in urothelial carcinoma of bladder and correlated their expression with various clinico-pathological variables like age, gender, tumor size, grade, stage, invasiveness of the tumor that might help in risk stratification and patient management. HER-2 NEU over expression can be a potential target in treating locally advanced or metastatic disease.

Aims and Objectives

AIMS & OBJECTIVES

- To evaluate the immunohistochemical expression of HER2 NEU, P53 & P63 in urothelial carcinoma of bladder.
- To correlate the immunohistochemical expression of these markers with various clinico-pathological variables like age, gender, tumor size, grade, invasiveness and stage of the tumor.



Review of Literature

REVIEW OF LITERATURE

EPIDEMIOLOGY:

Bladder cancer is the most common malignant tumor of the urinary system and transitional cell carcinoma accounts for more than 90% of all bladder tumors[24].Urothelial carcinoma of bladder is the fourth most common cancer in men and eighth most common malignancy in women in the western world[25]. As per the Indian cancer registry data,it is the ninth most common cancer and is three times more common in men than in women[26].

The Indian figures differ from the western literature in two aspects.First,the difference in the incidence of smoking among Indian males and females is much more prominent than in the west[27]. Second, the incidence of bladder cancer per se is much more predominant in Indian males. The higher incidence of bladder cancer in men versus women is explained by the smoking habits of men and estrogen-progesterone hormonal influence in the female reproductive life[28].

The incidence increases directly with age and the median age at diagnosis is around 50 years for each gender[29]. Age, gender and racial factors all affect the survival and prognosis of patients with bladder cancer[30].

A total of 40-45% of newly diagnosed bladder cancers are high grade lesions,more than half of which are muscle invasive at the time of diagnosis[31].The younger individuals present more frequently with low grade and low stage tumors than their elderly counterparts[32] and behave in an indolent fashion[33].

IMPACT OF BLADDER CANCER IN VARIOUS COUNTRIES:

Fig 1: Chart showing changing annual mortality rate of bladder cancer over a period of time.

From this chart, we infer the changing mortality rate in India relative to the parent region of South Asia and the world at large.

EMBRYOLOGY OF URINARY BLADDER

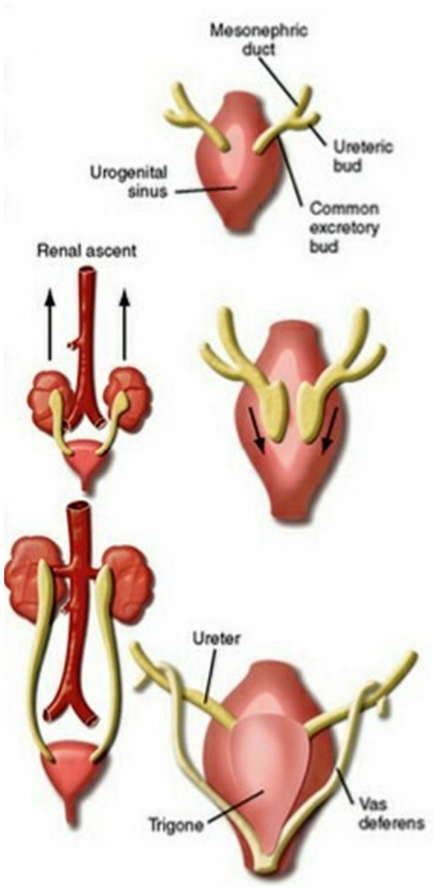


Fig 2: Showing development of urinary bladder and ascent of kidney.

The urinary bladder develops mainly from the vesical part of the urogenital sinus but its trigone is derived from the caudal ends of the mesonephric duct.The entire epithelium of the bladder is derived from the endoderm of the vesical part of the urogenital sinus and the other layers of its wall develop from adjacent splanchnic mesenchyme.

ANATOMY OF URINARY BLADDER

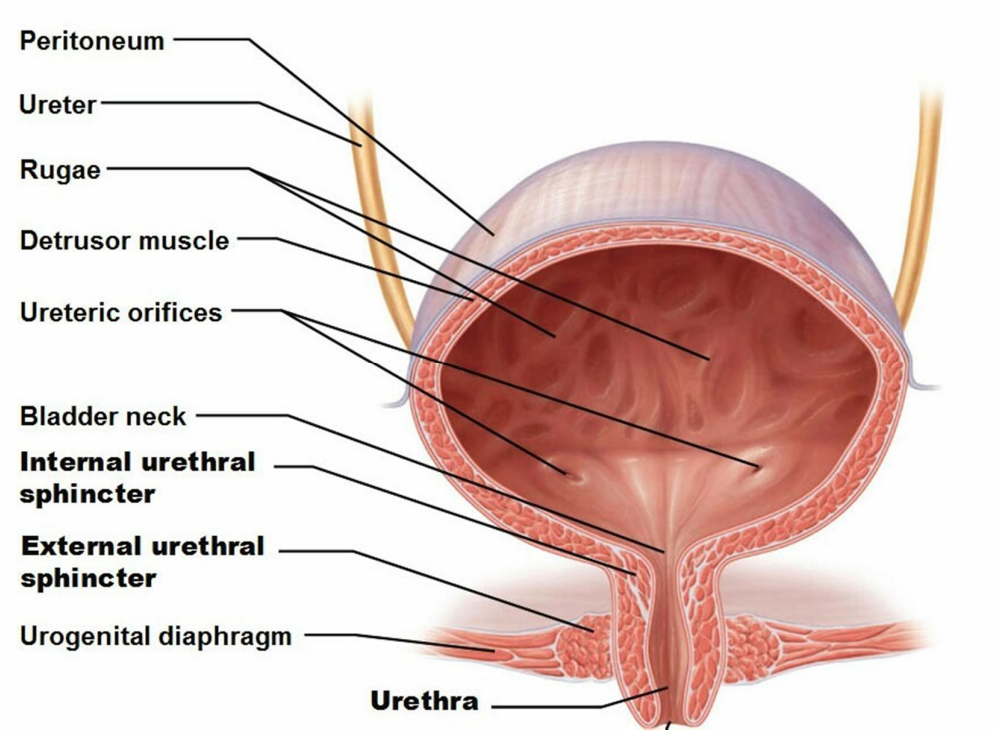


Fig 3: showing anatomy of urinary bladder

The bladder is a hollow viscus with the shape of a four sided inverted pyramid when empty and of a rounded structure when distended. It is divided into the following portions: superior surface (also known as dome, and covered by the pelvic parietal peritoneum),posterior surface (also known as base), and the two inferolateral surfaces. The trigone is located at the base of the bladder and is continuous with the bladder neck, in which the posterior and inferolateral walls converge to open into the urethera. The structure on which the bladder neck rests (rectum in males and vagina in females) is known as the bladder bed.

HISTOLOGY OF BLADDER

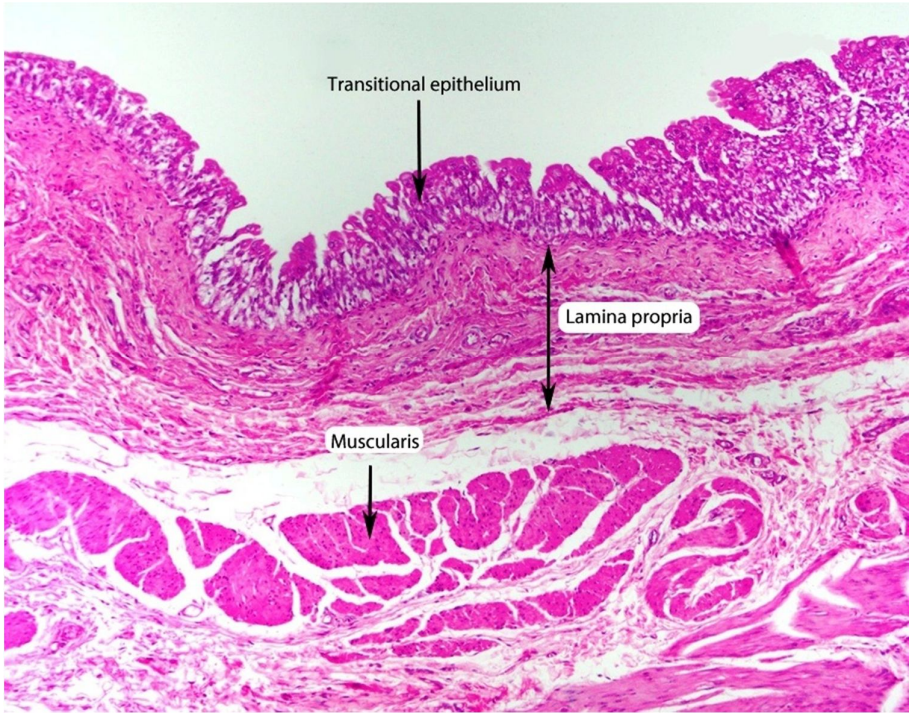


Fig 4: showing normal histology of urinary bladder.

The layers of the bladder are the mucosa, muscularis propria and adventitia. The latter is covered by serosa at the dome. The mucosa is formed by the epithelium,lamina propria and a continuous or discontinuous muscularis mucosae. The epithelium of bladder has been traditionally referred to as transitional, but the term urothelium is more informative and accurate.It is six to seven cells thick in the contracted bladder but only two or three cells thick in the distended bladder. It has three layers-superficial, intermediate and basal. The superficial layer is made up of a single row of large, elliptical cells having abundant eosinophilic cytoplasm and referred to as umbrella cells. The intermediate cells have cuboidal to low columnar shape, oval nuclei with finely stippled chromatin, moderately abundant cytoplasm and well defined margins.

The basal layer is made up of a row of cuboidal cells that lie on a thin continuous basal lamina. The lamina propria is composed of loose connective tissue containing a rich vascular network, lymph vessels and a few elastic fibres. The muscularis propria is vaguely divided into inner and outer longitudinal layers and a central layer located in between; these are best individualized in the bladder neck region.

RISK FACTORS FOR BLADDER CARCINOMA

The etiology of urothelial carcinoma is multifactorial in nature, although the world wide well recognized risk factors are cigarette smoking, occupational exposure to aromatic amines and urinary schistosomiasis.

TOBACCO SMOKING:

The most important risk factor for bladder cancer is tobacco smoking accounting for approximately 50% of the cases[34,35], because tobacco smoke contains aromatic amines and polycyclic aromatic hydrocarbons which are

excreted via kidney. A direct effect of cigarette smoke constituents and / or their metabolites on the bladder may be indicated by the presence of potent bladder carcinogen, 2-naphthylamine, in the smoke.[36]

Compared with nonsmokers, cigarette smokers have a two to four fold increased risk of bladder cancer[37] and the risk increases with increasing intensity and duration of smoking.[38] On cessation of smoking, the risk of bladder cancer falls by more than 30% after one to four years and by more than 60% after twenty five years but never returns to the risk level of nonsmokers. [39]

OCCUPATIONAL EXPOSURE:

Occupational exposure to various chemicals is the second most important risk factor for bladder carcinoma, accounting for about 10% of all cases. This type of occupational exposure occurs mainly in industrial plants processing paint, dye, metal and petroleum products.[34,40,41] The chemicals that are involved in bladder carcinogenesis include aromatic amines, polycyclic aromatic hydrocarbons and chlorinated hydrocarbons. Further evidence implicating various occupational exposure in the development of bladder cancer is reported in a study by Veys.[42] By reviewing detailed occupational histories of bladder cancer patients, Veys suggests that approximately 20% of those dying from bladder cancer may have had an occupational exposure related to tar, metal, dyes and rubber.

Case et.al. has showed an 18 year incubation period for bladder cancer after exposure to dyestuff chemicals, although apparently as little as 2 years exposure in high risk industries can initiate the development of bladder tumors.

INFECTION:

For more than half a century the relationship between infection by Schistosoma haematobium and bladder cancer has been known.[43] Although it is difficult to obtain the reliable incidence data of bladder cancer in endemic countries, the proportional incidence of bladder cancer appears to be high in these areas.[44]

Patients harboring Schistosoma haematobium infections are found to have squamous cell carcinoma of bladder rather transitional cell carcinoma. [34]

RADIATION:

Exposure to ionizing radiation is connected with increased risk. It is suggested that cyclophosphamide and pioglitazone are weakly associated with bladder cancer risk.[34]

COFFEE, ALCOHOL & TEA:

Over 30 studies have reported a higher risk (weak to moderate) for bladder cancer in coffee drinkers than in non-drinkers, but no trend with dose or duration [45]. It is still unclear whether the weak association is causal or nonspecific, or due to some bias or confounding variables[46]. High consumption of coffee (more than four cups per day) has been observed to increase bladder cancer risk. According to

epidemiological data, the association between alcohol consumption and bladder cancer risk is not well established, with most studies reporting a non significant association or no association [47,50]. Tea consumption is probably not associated with the occurrence of bladder cancer [50]. A weak inverse association between tea consumption and bladder cancer risk has been noted [48,49] but it is unclear whether it could be due to total fluid intake or to some specific tea compounds.

DIET:

High intake of fats, particularly animal fats, could increase the risk of bladder cancer [51-55]. Mutagens involved in bladder cancer etiology are probably formed during the heating process [56,57] from foods rich in fat or prepared in fat (fried foods).Products of protein pyrolysis (heterocyclic amines) and N-nitroso compounds could be synthesized during cooking or meat preservation [57,58,59]. Dietary intakes of grilled, salted and canned meat were associated with significantly increased risks of bladder cancer [59,60].

Most studies that investigated fruits and vegetables consumption reported an inverse relationship with bladder cancer – a lower risk with bladder cancer in subjects with high consumption. No significant association was found between total vegetable intake, vitamin A and vitamin C intake and bladder cancer and only a moderate inverse association with vitamin E intake [50,61]

DRUGS:

The increased risk of bladder cancer in young women who regularly used phenacetin-containing products remained present after adjustments for all other identified risk factors [(62)]. There are highly reliable and consistent data on cyclophosphamide therapy and the risk of bladder cancer [(63)]. Results from a population-based cohort study of patients with Wegener's granulomatosis indicated a dose-response relationship between cyclophosphamide and the risk of bladder cancer, and high cumulative risks in the entire cohort [(63)].

OTHER RISK FACTORS:

The relation between amount of fluid intake and bladder cancer is uncertain, the chlorination of drinking water and subsequent levels of trihalomethanes are potentially carcinogenic, while exposure to arsenic in drinking water increases the risk. [(34)]

An increased risk for bladder cancer has been suggested in users of permanent hair dyes with an NAT 2 slow acetylation phenotype. [(64,65)]

Finally, there is an increased evidence that genetic predisposition may influence the incidence of transitional cell carcinoma of the bladder, especially via its impact on susceptibility to other risk factors. [(34,66)]

WHO / ISUP CLASSIFICATION SYSTEM-2016

The fourth edition of the WHO classification of tumors of the urothelial tract (2004) provides a contemporary review of the morphology of urothelial neoplasms, emphasizing their unique ability to exhibit divergent differentiation, morphological variants, and a diverse genomic landscape [(67)]. Grading of urothelial tumors has particular importance in non invasive disease, specifically papillary neoplasms. Although a small percentage of invasive carcinomas are low grade, usually limited to the lamina propria, more than 95% of invasive tumors are high grade.

As in 2004, the 2016 WHO classification continues to recommend the application of the grading classification first put forth by ISUP in 1997[(68)]. In fact, this classification continues to be endorsed by ISUP and all major contemporary pathology textbooks and guidelines.

In 2016, the newly described or better defined non invasive urothelial lesions include urothelial dysplasia and urothelial proliferation of uncertain malignant potential, which is frequently identified in patients with a prior history of urothelial carcinomas. Invasive urothelial carcinoma with divergent differentiation refers to tumors with some percentage of “usual type” urothelial carcinoma combined with other morphologies and percentage of this divergent histology has to be mentioned in the report [(68)].

GENOMICS OF BLADDER CANCER:

Various studies have suggested that there are two molecular pathways that lead to the development of invasive urothelial carcinomas. There is a difference in the molecular alteration between low and high grade tumors and between those that are invasive and those that are not. The increasing tumor grade and stage have been associated with copy number abnormalities, loss of heterozygosity and increased genomic instability. Multiple tumor suppressor genes and oncogenes have been described in invasive urothelial carcinoma, but it is difficult to determine whether all these are required for tumor development [69,70] .

The most common mutation in urothelial carcinoma involves TP 53 and FGFR 3 gene along with promoter mutations of TERT [71,72,73]. Other genes include PIK3CA, RB1, H-RAS.

Almost 79% of bladder neoplasm harbor TERT mutation, and they have no association with clinical outcome; however, its presence can be of diagnostic utility; given the relative rarity of this mutation in other tumors that may have overlapping histology.

The most frequently altered molecular pathways in urothelial carcinoma include the PI3K/AKT/ mammalian target of rapamycin pathway[74-77], the FGFR3/RAF/RAS pathway, the TP 53/ RB 1 pathway, immune response checkpoint modulators[78,79], and chromatin regulating & remodeling genes[80,81,82]. These pathways are mutually exclusive and some components of these pathways

are altered in low risk disease, whereas others are characteristic of high risk disease.

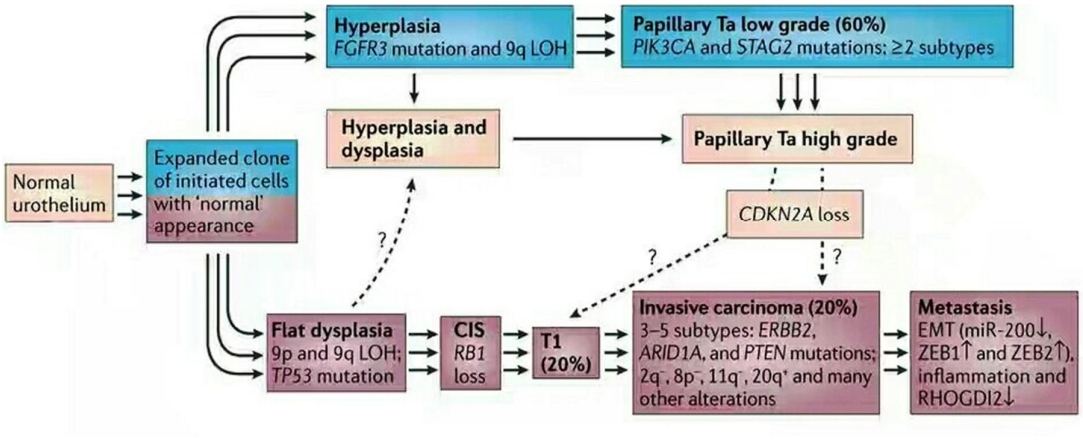


Fig 5: showing molecular pathogenesis of urothelial carcinoma

About 80% of papillary non invasive and low grade carcinoma harbor FGFR 3 mutations. Although these mutations have been associated with a higher risk of recurrence, they are not associated with disease progression [(83)]. Most muscle invasive bladder tumors have mutation in chromatin remodeling and histone modifying genes [(84,85)]. The genes involved in these pathways are targeted by novel therapeutic agents and thus patients can benefit from this targeted therapy. In addition, emerging data show that immune modulating agents may have a promising role in the management of advanced urothelial carcinoma. The molecular pathways that are involved in urothelial cancer recurrence and progression has allowed for the identification of potential prognostic and predictive markers [(84,86,87)] and it has led to the development of novel noninvasive

detection and surveillance strategies and revealed potential therapeutic targets[88-93].

Thus this study is performed to assess the immunohistochemical expression of various markers which are involved in the above mentioned molecular pathways and correlating their expression with various clinico-pathological variables like age, gender, size, tumor grade, stage and invasiveness of the tumor.

DIAGNOSTIC MODALITIES OF BLADDER CARCINOMA:

Various diagnostic modalities are available for detecting bladder cancer. These include invasive and non invasive methods. The single most reliable method is cystoscopy and helps in diagnosing both papillary and sessile urothelial lesion [94]. However, it is invasive and a source of distress for patients. It has a limited ability to detect occult microscopic disease or the presence of tumors in atypical locations.

URINE CYTOLOGY:

It is the simplest non invasive method for detecting bladder cancer. Urinary cytology helps in identifying the malignant cells that have been exfoliated from the urothelium into the urine. The specificity of cytology is greater than 90% [95], while the sensitivity for high grade disease and carcinoma in situ can be as high as 80 to 90% [96,97]. The main drawback of voided cytology is the low sensitivity (approximately 20-50%) for detecting low grade neoplasm and low grade papillary urothelial carcinoma [98,99,100]. The two main reasons for such low

sensitivity are cohesive nature of the malignant cells and they have similar cytomorphology to normal urothelial cells microscopically. Thus, voided urine cytology is a useful non invasive adjunct to cystoscopy because of its overall high specificity.

MORPHOLOGICAL CLASSIFICATION OF UROTHELIAL TUMORS:

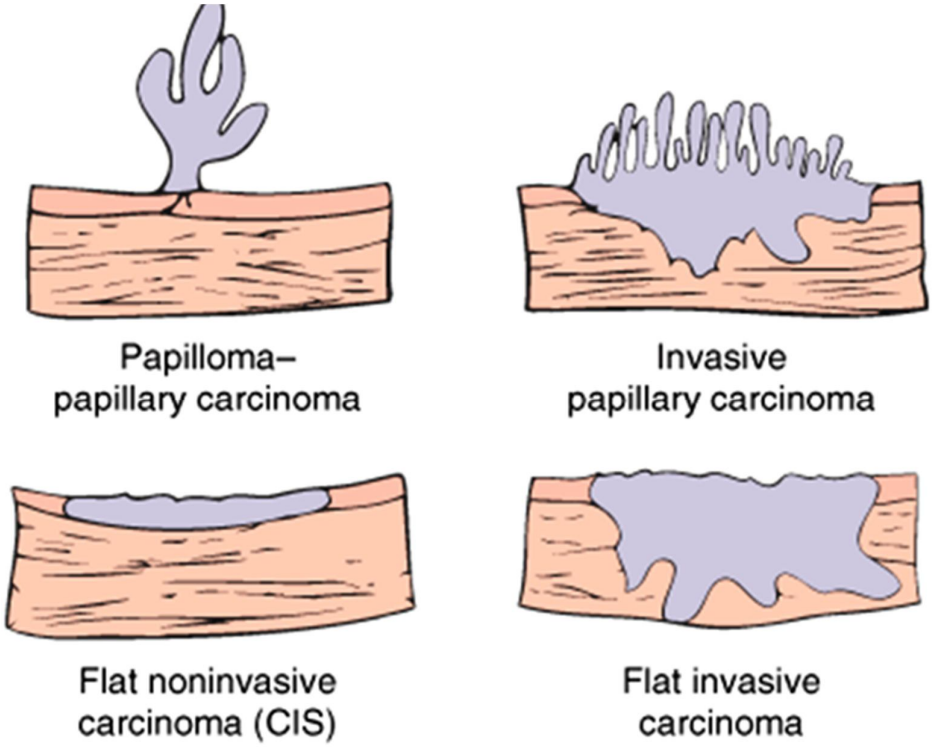


Fig 6: showing morphological types of urothelial carcinoma.

The urothelial neoplasm includes a spectrum of lesions ranging from noninvasive carcinoma (carcinoma in situ) to invasive carcinoma which may have flat to papillary configuration. Based on invasiveness, we can categorize them as muscle invasive and non muscle invasive tumors.

HISTOLOGICAL TYPING OF UROTHELIAL NEOPLASM:

These include flat urothelial lesions with atypia, papillary urothelial neoplasms, invasive urothelial neoplasms with divergent differentiation, squamous neoplasms, and glandular neoplasms.

FLAT UROTHELIAL LESIONS WITH ATYPIA:

These include reactive atypia, urothelial dysplasia, and urothelial carcinoma in situ.

UROTHELIAL DYSPLASIA:

Dysplasia is an intraepithelial neoplastic proliferation of urothelial cells characterized by variable degrees of loss of polarity, nuclear enlargement and chromatin clumping, all of which fall short of the degree seen in carcinoma in situ.

UROTHELIAL CARCINOMA IN SITU:

This is a high grade, often multifocal intraurothelial neoplastic proliferation characterized by unequivocal malignant urothelial cells within the bladder epithelial lining and does not involve the entire thickness of the urothelium. The dyscohesive nature of the cells often leads to denudation and a clinging pattern of growth in which only scarce malignant cells remain attached to the bladder wall.

PAPILLARY UROTHELIAL NEOPLASMS:

UROTHELIAL PAPILLOMA:

It is a benign neoplasm composed of delicate papillary fronds with no or minimal branching or fusion. The neoplastic cells are identical to normal urothelial cell and mitosis is absent.

INVERTED PAPILLOMA:

It is composed of anastomosing islands and cords of bland urothelial cells that invaginate and grow downward in the lamina propria with peripheral palisading, absent to rare mitosis, and absent to minimal cytological atypia.

PAPILLARY UROTHELIAL NEOPLASM OF LOW MALIGNANT POTENTIAL:

Histologically, it is characterized by orderly arranged and fused papillae exhibiting nuclei which is larger than that seen in papillomas. Mitotic figures are rare and confined to the basal layer.

NONINVASIVE PAPILLARY UROTHELIAL CARCINOMA, LOW GRADE:

The papillae are fused and show frequent branching with variations in nuclear size, shape and contour. Mitoses are occasional and may be found at any level.

NONINVASIVE PAPILLARY UROTHELIAL CARCINOMA, HIGH GRADE:

They are characterized by frequent branching and fusion with moderate to marked cytoarchitectural disorder and nuclear pleomorphism. Mitoses are frequent and they progress to invasive carcinoma.

INVASIVE UROTHELIAL CARCINOMA:

The malignant urothelial cell infiltrates the various layers of the bladder wall and is the single most important prognostic factor.

Numerous histological variants of urothelial carcinoma with divergent differentiation have been described and these include invasive urothelial carcinoma with squamous differentiation, invasive urothelial carcinoma with glandular differentiation, urothelial carcinoma with trophoblastic differentiation. Other variants include sarcomatoid variant, nested variant, microcystic variant, micropapillary variant, lymphoepithelioma like variant, plasmacytoid variant, giant cell variant, glycogen rich variant and urothelial carcinoma with rhabdoid features.

STAGING AND GRADING OF BLADDER CANCER:

Grading of urothelial tumors has particular importance in noninvasive disease, specifically papillary neoplasms. Inter observer variability is high, even among experienced pathologists, despite many decades of efforts to develop pathological classifications that best reflect clinical behavior [101-111]. Multiple studies have been published comparing this classification with others, particularly the 1973 WHO classification, in terms of reproducibility and clinical impact.

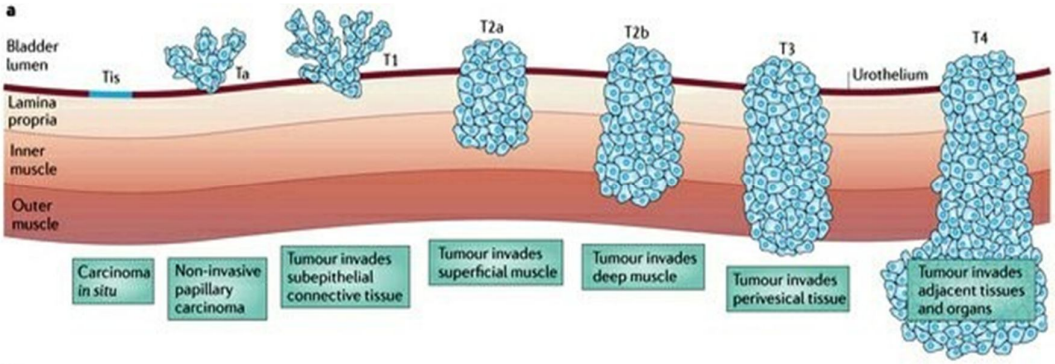


Fig 7: showing staging of urothelial carcinoma

The staging of bladder cancer mainly depends on the depth of invasion and is the single most important determinant for patient survival.

PROGNOSTIC FACTORS:

The prognostic factors that predict recurrence and progression are divided into three groups and categorized based on clinical, endoscopic and pathological findings. [112-119]

1. **Prognostic factors based on clinical features:**
    - Primary or recurrent tumor
    - Prior recurrence rate
    - Use of intravesical therapy.

2. **Prognostic factors based on endoscopic findings:**
    - Number of tumor
    - Tumor size

3. Prognostic factors based on pathological findings:

- Tumor grade
- Tumor stage
- Association with carcinoma in situ.

Type of tumor:

The prognosis is different for each type of bladder cancer. Papillary urothelial carcinomas of the bladder have the best prognosis. Small cell carcinoma tends to have a poor prognosis.

Stage:

It is the most important prognostic factor. The lower the stage, the more favorable the prognosis. The deeper the cancer has grown into the bladder wall or surrounding tissue, the less favorable the prognosis. Cancer that has spread to the lymph nodes or to other areas of the body has a poorer prognosis.

Grade:

The lower the grade, the more favorable the prognosis. High grade tumors have a greater risk of disease progression and a less favorable prognosis.

Tumor size:

People with smaller tumors have a more favorable prognosis than people with large tumors.

Recurrence rate and time to recurrence:

Recurrent tumors have poor prognosis and the time to recurrence is also an important prognostic factor. Tumors that recur within the first two years after diagnosis and successful treatment are more aggressive and have a higher chance of distant metastasis.

Recent predictive and prognostic markers involved in the molecular pathogenesis are under study. In this study, we analyzed the immunohistochemical expression of HER 2 NEU, P53, P63 and correlated their expression with various clinico-pathological variables like age, gender, size of the tumor, grade, stage and invasiveness of the tumor.

IMMUNOHISTOCHEMISTRY:

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. In 1966, Nakane and Pierce et al introduced the indirect labeling technique in which the unlabelled antibody is followed by second antibody or substrate. Various immunohistochemical methods include peroxidase-anti peroxidase method (1970), alkaline phosphatase labeling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993).

ANTIGEN RETRIEVAL:

To unmask the antigenic determinants in fixed tissue sections, following methods are used:

1. Proteolytic enzyme digestion.
2. Microwave antigen retrieval.

3. Pressure cooker antigen retrieval.
4. Microwave and trypsin antigen retrieval.

PROTEOLYTIC ENZYME DIGESTION:

In 1976, Huank et al introduced this technique and it depends on the breakdown of formalin cross linkages. The most commonly used enzymes include trypsin and proteinase. The disadvantages include over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This technique is being practiced in various institutions as it allows rapid and uniform heating of the paraffin sections.

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method. [120]

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat treatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pretreatment and some antigens show altered staining pattern.

DETECTION SYSTEMS:

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect method.

In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are fluoro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains.

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method [(121)].

Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

UTILITY OF IHC IN UROTHELIAL LESIONS:

Role of immunohistochemistry in flat lesions:

Immunohistochemistry plays an important role in distinguishing reactive urothelial cells from dysplastic urothelial cells / carcinoma in situ lesions. Cytokeratin 20 (CK 20) is the marker that distinguishes these lesions. CK 20 is

positive only in the umbrella cells of the reactive urothelium whereas dysplastic/ carcinoma in situ cells show full thickness increased expression. As both dysplastic cells and carcinoma in situ cells express full thickness CK 20, it is not useful in differentiating these lesions and the morphology plays a crucial role in differentiating them. Other markers that help in differentiating reactive urothelial cells from dysplasia/ carcinoma in situ include CK 5/6, P16, Ki 67. CK 5/6 shows diffuse strong positivity in reactive cells whereas dysplastic cells express only in basal layer or show negative staining pattern. P16 shows strong positivity in carcinoma in situ whereas negative in reactive cells. Carcinoma in situ shows increased Ki 67 index whereas it is low in reactive cells.

Role of immunohistochemistry in urothelial carcinoma:

Most urothelial carcinoma express both CK 7 & CK 20 whereas primary adenocarcinoma of bladder express CK 20 only. Urothelial differentiation markers include uroplakin, GATA 3, P63. In addition, immunohistochemistry aids in differentiating primary urothelial carcinoma from renal cell carcinoma, prostatic carcinoma and nephrogenic adenoma.

In the present series, we evaluated the immunohistochemical expression of HER 2 NEU, P53, P63 and correlated their expression with various clinico-pathological variables that might predict patient outcome and further management.

HER 2 NEU:

The HER 2 NEU gene is located on chromosome 17q and encodes for a tyrosine kinase receptor which is thought to control cell growth and development[122,123]. Its activation increases the mitotic activity and metastatic potential of the cell leading to oncogenic transformation. HER 2 NEU over expression and amplification was first identified in a human breast cancer cell line[124]. It has been considered as a prognostic marker in breast carcinoma, particularly in lymph node positive cases[125,126,127]. The over expression of HER 2 NEU have been observed in various organs like stomach, colon, bladder, prostatic gland, salivary gland, ovary and uterus. Since the important prognostic and therapeutic impact that HER 2 NEU status had in breast carcinoma, more interest has been given to its expression in other cancers.

In 1990, Zhau et al, first reported an increased amplification and an over expression of HER 2 NEU in bladder cancer [128]. Since then, several studies have been conducted and the over expression of HER 2 NEU in urothelial carcinoma ranged between 17% and 76% of invasive carcinoma [129].

The prognostic impact of HER 2 NEU on urothelial carcinoma is variable among several studies. Various studies (Jamez et al, Under wood et al, Kringer et al) found that HER 2 NEU over expression is predictive of bladder cancer death in patients with invasive cancer (130,131). B.Kolla et al observed a significantly high disease free survival in HER 2 NEU negative patients compared to HER 2

NEU positive patients; this difference was more profound in patients with locally advanced disease.

Recently, an anti HER 2 NEU antibody was proposed as therapeutic tool in invasive bladder cancer and the response rate ranges from 3 to 63% [132]. Thus, a reliable evaluation is needed to introduce targeted therapy in the management of invasive urothelial carcinoma.

Various studies on HER 2 NEU over expression:

STUDIES	NUMBER OF CASES	INVASIVENESS OF THE TUMOR	PERCENTAGE OF CASES WHICH SHOWED HER 2 NEU OVER EXPRESSION
Mejri et.al, 2014	21	MI	45%
Jimenez et.al, 2001	80	MI	28%
Nedjadi et.al, 2016	160	MI	25%
Edwards et.al, 2002	39	MI	71%
Santhosh et.al, 2012	100	MI	70%
Charfi et.al, 2013	151	MI/NMI	9.3%

P53:

P53 was identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine and Lloyd Old. The human TP 53 gene was cloned in 1985. Its role as a tumor suppressor gene was discovered in 1989 by Bert Vogelstein. It is considered as “Guardian of the genome” and its gene is located on the 17p

chromosome, coding a protein of 53 Kd. This protein is encoded by the gene TP 53. The role of P53 is central in cell-cycle regulation, in DNA repair and in cell apoptosis. The increased P53 production occurs in response to cellular insults or DNA damage and then it induces arrest of cell cycle at the G1/S junction[133,134]. Therefore, P53 is essential for control of tumor growth, apoptosis and maintaining genome stability. Unlike normal P53 protein, which is rapidly removed from the nucleus, mutant forms have a prolonged half life, which favors intranuclear accumulation and this can be detected by immunohistochemistry.

Mutation of the P53 gene has been observed in a wide variety of human carcinomas, such as lung carcinoma, colorectal carcinoma, oropharyngeal carcinoma, breast carcinoma, gall bladder carcinoma, bladder carcinoma and gastric carcinoma [135]. Numerous studies have reported the correlation between the over expression of P53 and the poor prognosis of patients with these tumors. The P53 pathway is also involved in regulating the metastasis- associated genes, including Maspin, integrin, matrix metallo-proteinase-2 (MMP-2), MMP-13 and the tissue inhibitor of metalloproteinase-2 (TIMP 3).

Several studies have documented the P53 mutations in urinary bladder carcinoma and the frequency with which these mutation was found were between 6% and 61% (Shipman et al, 1997, Sidransky et al, 1991).

Various studies on P53 over expression [136,137]:

STUDIES	MATERIALS	GRADE	PERCENTAGE OF OVER EXPRESSION
Koyuncuer et al, 2017	62	High and low	62.9% of high grade invasive carcinoma showed more than 10% over expression
Grapsa et al, 2014	100	High	26%
Kuczyk et al, 1995	44	High	70%
Teng A. Ong et al,2000	64	High and low	87.5% of high grade carcinoma demonstrated P53 over expression

P63:

The P63 gene is a homologue of the P53 tumor suppressor gene located at 3q27-3q29 and encodes multiple proteins that may either trans activate P53 responsive genes or act as a dominant negative factor towards P53 and P63 [138]. P63 is a nuclear marker and it is expressed in the basal cells of stratified epithelium, including the urothelium [139] and plays a critical role in the normal development and maintenance of the human urothelium [140]. In the absence of P63, a cuboidal epithelium will be formed that lacks the morphological characteristics of a transitional epithelium. P63 is also considered as a myoepithelial marker and it helps in assessing tumor invasion in breast carcinoma and salivary gland carcinoma.

Various studies demonstrated that P63 is down regulated in muscle invasive bladder cancers and others proposed an impaired expression with

biological aggressiveness, a feature of high grade carcinoma, suggesting a role in tumor progression and biochemical differentiation [141,142].

Several studies on P63 expression: [136,143,144]

STUDIES	TOTAL NO OF CASES	NO OF CASES EXHIBITING DECREASED P63 EXPRESSION	PERCENTAGE OF CASES
Koyuncuer et al, 2017	151	58	38%
Urist et al, 2002	160	108	68%
Mursi et al,2013	25	16	64%
Fumitaka Koga et al,2015	75	39	52%

Materials and Methods

MATERIALS AND METHODS

In this study, we performed both prospective and retrospective data analysis of patients who were diagnosed to have biopsy proven urothelial carcinoma over a period of two years from June 2015 to June 2017 in Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai.

During our study period, we received 22,178 specimens for histopathological examination. Of the total 22,178 specimen, 423 cases belong to urinary system which includes nephrectomy specimen accounting for 300 cases and indications for nephrectomy include both neoplastic and non neoplastic lesions of kidney. 123 cases of bladder specimen were received and most of them were clinically suspected to have bladder carcinoma. Out of 123 bladder specimen, radical cystectomy specimen accounted for 37 cases, and remaining 86 were TURBT specimen.

All 123 bladder specimen were subjected for histopathological examination and 46 turned to be high grade urothelial carcinoma (39 muscle invasive and 7 non muscle invasive), 38 cases diagnosed as low grade urothelial carcinoma (9 muscle invasive and 29 non muscle invasive), 32 cases showed features of benign and non neoplastic conditions and conclusive opinion could not be arrived in 7 cases because of sampling error.

INCLUSION CRITERIA:

All cystectomy and trans uretheral resection of bladder specimen that were histologically diagnosed as urothelial carcinoma irrespective of age, gender, grade and stage was included in the study.

EXCLUSION CRITERIA:

- Benign urothelial lesions.
- Non specific inflammatory conditions.
- Other histological variants of urothelial carcinoma like micropapillary variant, urothelial carcinoma with squamoid differentiation and squamous cell carcinoma of bladder were excluded from the study population.
- Lack of paraffin blocks with representative tumor tissue was excluded from the study population.

METHOD OF DATA COLLECTION:

Detailed history of the cases regarding age, sex, personal history, site, type of procedure done were obtained for all 123 cases reported during the study period from surgical pathology records. All trans uretheral resection of bladder specimen were processed entirely and representative sections were taken from radical cystectomy specimen which were subjected for routine histopathological examination. The following clinical and pathological parameters were evaluated: age, gender, tumor site, size, tumor grade, tumor stage and invasiveness of the tumor.

Urothelial carcinoma was graded as high grade and low grade based on architectural distortion, cytological and nuclear atypia. Further based on the invasiveness, it was subclassified as muscle invasive and non muscle invasive bladder carcinoma. Among 123 cases, equal proportion of high and low grade urothelial carcinoma were selected randomly and 26 low grade, 26 high grade urothelial carcinoma were included. Out of 26 high grade carcinoma, 24 were muscle invasive and 2 were non muscle invasive. Among 26 low grade urothelial carcinoma, 5 were muscle invasive and 21 were non muscle invasive carcinoma. These 52 cases were analyzed for immunohistochemical expression of P53, P63 and HER 2 NEU.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemical analysis of HER 2 NEU, P53 & P63 were performed in paraffin embedded tissue samples using super sensitive polymer HRP system based on non biotin polymeric technology. 4 micron sections were cut from formalin fixed paraffin embedded tissue samples and transferred onto positively charged slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (Pathnsitu) against P53, P63 protein & rabbit monoclonal antibody against HER 2 NEU and then detected by adding secondary antibody conjugated with horse radish peroxidase-polymer and diaminobenzidine substrate.

ANTIGEN	VENDOR	SPECIES (CLONE)	DILUTION	POSITIVE CONTROL
HER 2 NEU	PATHNSITU	RABBIT	READY TO USE	BREAST CARCINOMA
P53	PATHNSITU	MOUSE	READY TO USE	COLON ADENOCA
P63	PATHNSITU	MOUSE	READY TO USE	SCC-ORAL CAVITY

INTERPRETATION AND SCORING SYSTEM:

The antibody treated slides were analyzed for the presence or absence of reaction, localization of the staining pattern, percentage of cells stained and intensity of the reaction.

EVALUATION OF HER 2 NEU STAINING:

For assessing HER 2 NEU positivity, ASCO scoring system was used. According to this system, only membranous staining pattern was considered positive and the level of HER 2 NEU expression was assessed semi-quantitatively by the intensity & percentage of cells stained and scored on a scale of 0-3+ [(145)]. A cytoplasmic staining was considered nonspecific.

ASCO SCORING FOR HER 2 NEU EXPRESSION:

Score	**Localization**	**Intensity**	**Percentage of cells stained**
Score 0	Nil	Nil	No cell stained
Score 1+	Membranous	Barely perceptible	More than 10%
Score 2+	Membranous	Weak to moderate complete membranous staining	More than 10%
Score 3+	Membranous	Strong complete membranous staining	More than 30%

EVALUATION OF P53 STAINING:

P53 is a tumor suppressor protein and nuclear staining was considered positive. The criteria for P53 over expression is that more than 10% of the tumor cells should exhibit nuclear positivity, [146] in accordance with the practice used in previous studies because it has been shown that nuclear positivity in more than 10% of the tumor cells correlates with mutations in the P53 gene.

EVALUATION OF P63 STAINING:

The cut off for decreased expression of P63 is that less than 90% of the tumor cells exhibiting nuclear positivity and staining of more than 90% of cells is considered as normal expression of P63 [147].

CRITERIA FOR P53 & P63 EXPRESSION:

Marker	**Localization**	**Expression pattern**	
P53	Nuclear	More than 10% of cells stained-over expression	Less than 10% of cells stained-negative staining
P63	Nuclear	Less than 90%of cells stained-decreased expression	More than 90% of cells stained-normal staining pattern

STATISTICAL ANALYSIS

The statistical evaluation was performed with IBM-SPSS statistical package for the social sciences version 20. An initial analysis of collected variables was performed. Immunohistochemical expression of HER 2 NEU, P53, P63 were analyzed and correlated with clinical variables like age, gender, size and pathological variables like histological grade, stage and invasiveness of the tumor. Pearson Chi square test was used in analyzing these variables.

Immunohistochemical expression of HER 2 NEU was compared with P53 and P63 expression. Similarly P53 expression is compared with P63 expression and analyzed for statistical correlation. In the present study, the P value below 0.05 is considered significant.

Observation and Results

OBSERVATION AND RESULTS

During the study period of 24 months from June 2015-June 2017, a total of 22,178 specimens were received in the Institute of Pathology, Madras Medical College for histopathological examination. Of the total cases, 123 bladder specimens were received and this included both Transuretheral resection of bladder tumor (86 specimen) and 37 radical cystectomy specimen. Among 123 cases, 84 were reported as urothelial carcinoma (46 high grade and 38 low grade), 32 were diagnosed as non neoplastic and benign lesions and conclusive opinion could not be arrived in remaining cases because of inadequate sampling.

Table 1: Total number of urothelial carcinoma diagnosed during study period:

Grade	Muscle invasive	Non muscle invasive	Total
High	39 (85%)	7 (15%)	46 (55%)
Low	9 (24%)	29 (76%)	38 (45%)
Total	48	36	84 (100%)

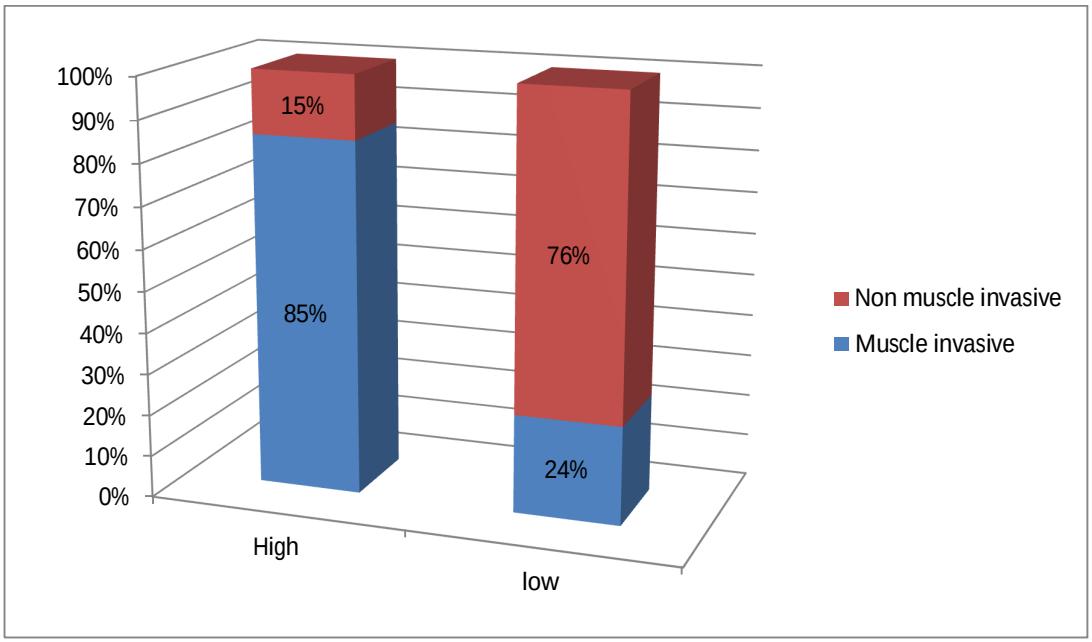


Chart 1: showing percentage of muscle invasive and non muscle invasive carcinoma among 84 urothelial carcinoma cases.

Among 46 high grade carcinoma (55%), 85% constituted muscle invasive (39 cases) and 15% were non muscle invasive (7 cases). Out of 38 low grade carcinoma (45%), 24% were muscle invasive (9 cases) and 76% were non muscle invasive (29 cases). Thus, we inferred from this study that the incidence of high grade urothelial carcinomas outnumbered low grade carcinomas and most high grade carcinomas were muscle invasive and low grade carcinomas were non muscle invasive. Muscle invasion was more frequently encountered in high grade than in low grade carcinomas.

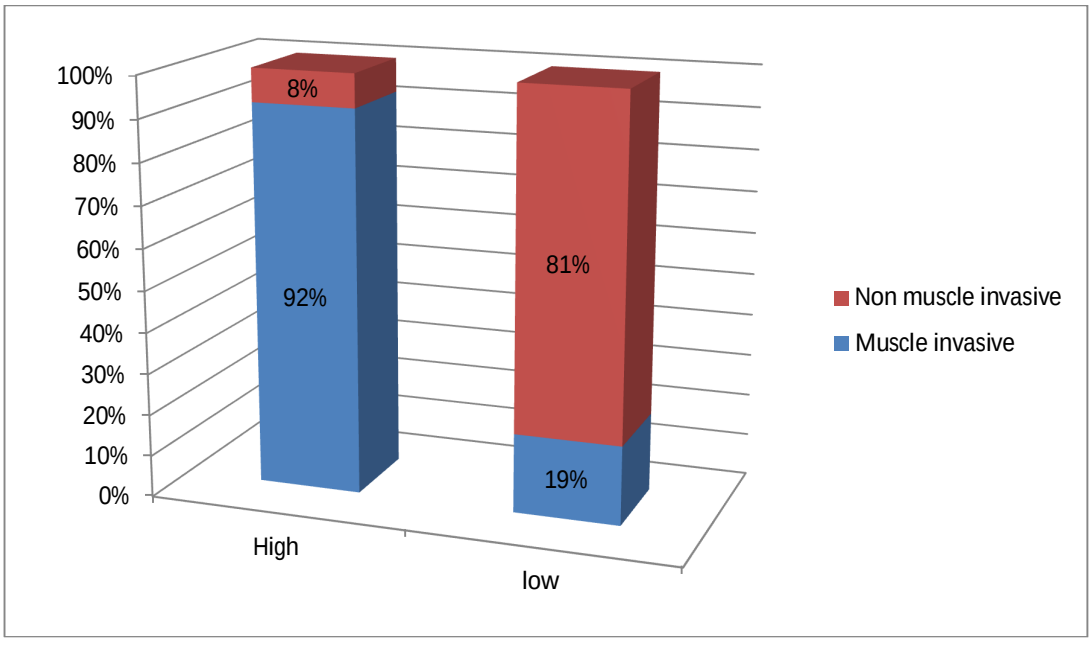
Other histological variants like micropapillary variant, primary squamous cell carcinoma of bladder, urothelial carcinoma with squamoid differentiation have been reported in our Institute.

Among 84 cases of urothelial carcinoma, 52 cases were selected based on availability of tissue block and clinical data. Equal proportion of high grade and low grade carcinomas were taken into account for easy comparison. However, the frequency with which muscle invasion occurs was more in high grade than in low grade carcinoma. Thus an equal number of muscle invasive and non muscle invasive tumors were not taken into account.

Table 2: Number of high grade and low grade carcinoma in the study population:

Grade	Muscle invasive	Non muscle invasive	Total
High	24 (92%)	2 (8%)	26 (100%)
Low	5 (19%)	21 (81%)	26 (100%)
Total	29 (56%)	23 (44%)	52 (100%)

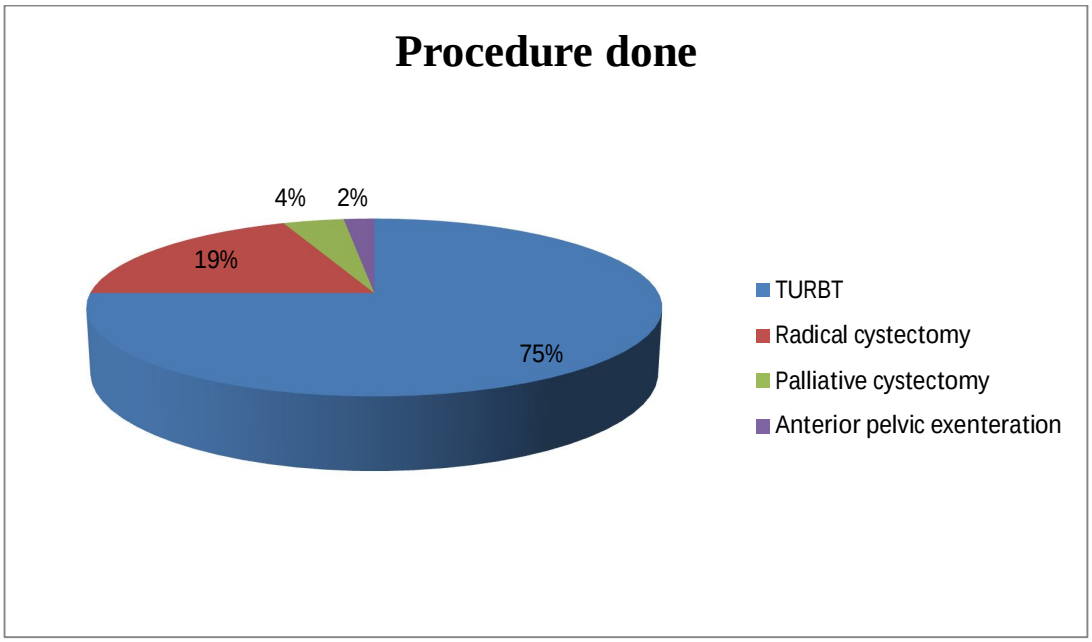
Chart 2: showing percentage of muscle invasive and non muscle invasive carcinoma among the study population (52 cases).



Among 52 cases in the study population, 50% were high grade (26 cases) and 50% were low grade urothelial carcinoma (26 cases). About 92% of high grade carcinomas were muscle invasive and 81% of low grade carcinomas were non muscle invasive type.

TYPE OF SPECIMEN RECEIVED:

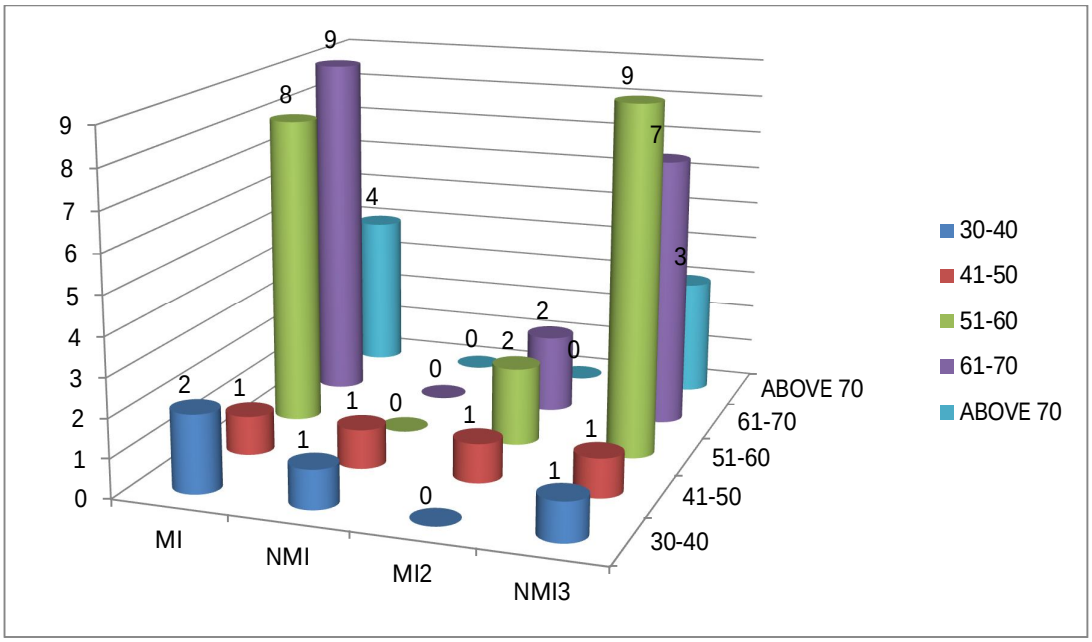
Chart 3: showing type of specimen received among the study population (52 cases).



In the study population, 75 %were TURBT specimen (39 cases) and remaining 25% were resected specimen (13 cases). TURBT was performed more frequently than bladder resection because most patients present with advanced stage and thus palliative treatment was the main modality of treatment. As bladder carcinoma was more common in older age group, the morbidity and mortality related to surgery was high and thus TURBT was performed more frequently.

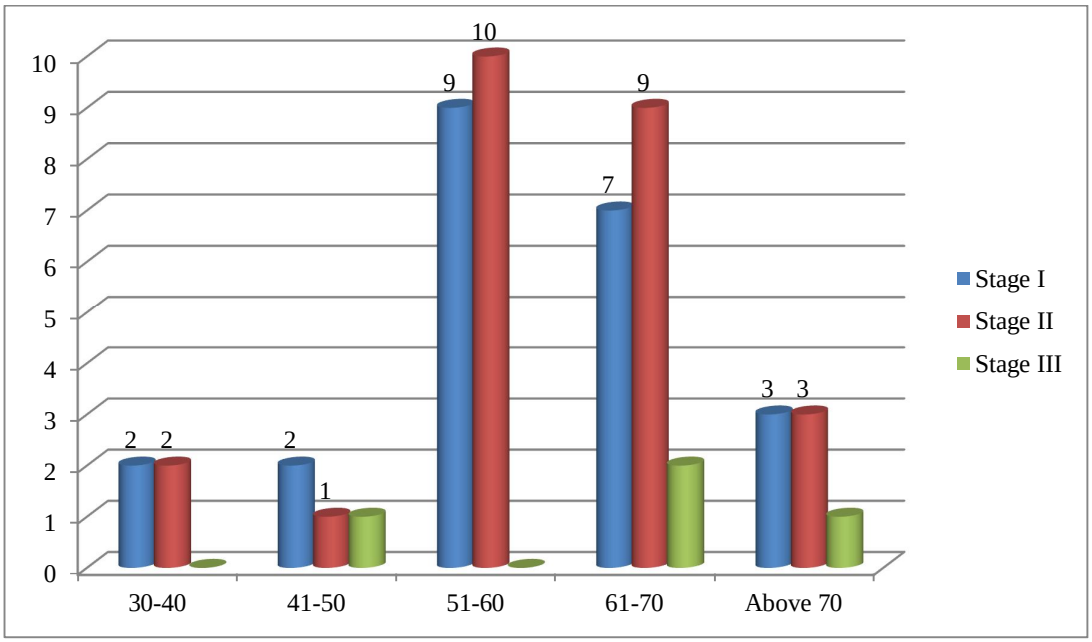
AGE WISE DISTRIBUTION OF CASES:

Chart 4: showing incidence of muscle invasive and non muscle invasive carcinoma among various age group.



In this study, the peak incidence of urothelial carcinoma occurred above 50 years of age. The mean age group for high grade carcinoma was 61-70 years and for low grade carcinoma was 51-60 years. It is inferred that as age advances, the tumor grade is higher.

Chart 5: showing number of cases among various stage with in each age group:



From the above chart it was clear that as age advances, the stage of the tumor was high and might affect the outcome of the patient.

GENDER WISE DISTRIBUTION OF CASES:

Table 3: showing gender wise distribution of cases

		GRADE				
		HIGH		LOW		TOTAL
		INVASIVENESS		INVASIVENESS		
		MI	NMI	MI	NMI	
SEX	FEMALE	9	0	1	6	16
	MALE	15	2	4	15	36
	Subtotal	24	2	5	21	52

In the present series, males constituted 69% (36 cases) and females accounted for 31% (16 cases) of urothelial carcinoma. Among high grade carcinoma, males constituted 65% (17 cases) and females accounted for 35% (9 cases) of cases. In low grade carcinoma, males constituted 73% (19 cases) and females accounted for 27% (7 cases). Thus the overall incidence of urothelial carcinoma was higher in males than in females.

SITE WISE DISTRIBUTION OF BLADDER CARCINOMA:

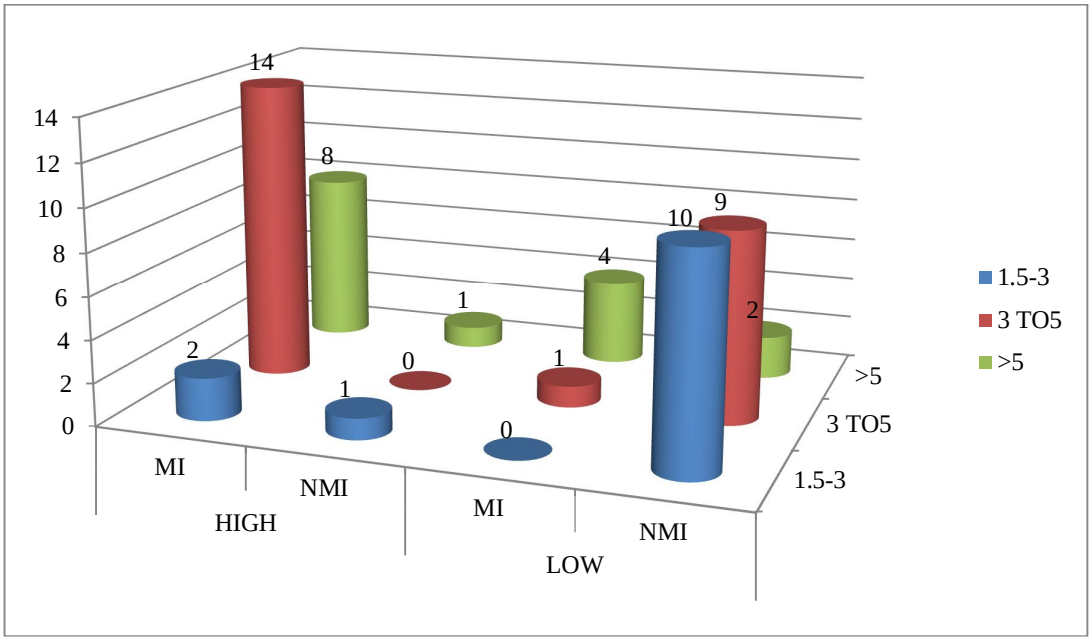
Table 4: showing site wise distribution of urothelial carcinoma:

SITE OF GROWTH	GRADE				Total
	HIGH		LOW		
	INVASIVENESS		INVASIVENESS		
	MI	NMI	MI	NMI	
	Count	Count	Count	Count	
ANTERIOR WALL	0	1	0	0	1
ANTEROLATERAL WALL	1	0	0	0	1
LATERAL WALL	12	1	5	14	32
LATERAL WALL,BASE	1	0	0	1	2
POSTERIOR WALL	0	0	0	2	2
POSTEROLATERAL WALL	10	0	0	3	13
TRIGONE	0	0	0	1	1

In this study, the most common site of urothelial carcinoma was lateral wall (32 cases) followed by posterolateral wall (13 cases). A statistical analysis was performed between site of the tumor and grade (P value=0.070) and no correlation was observed between these variables.

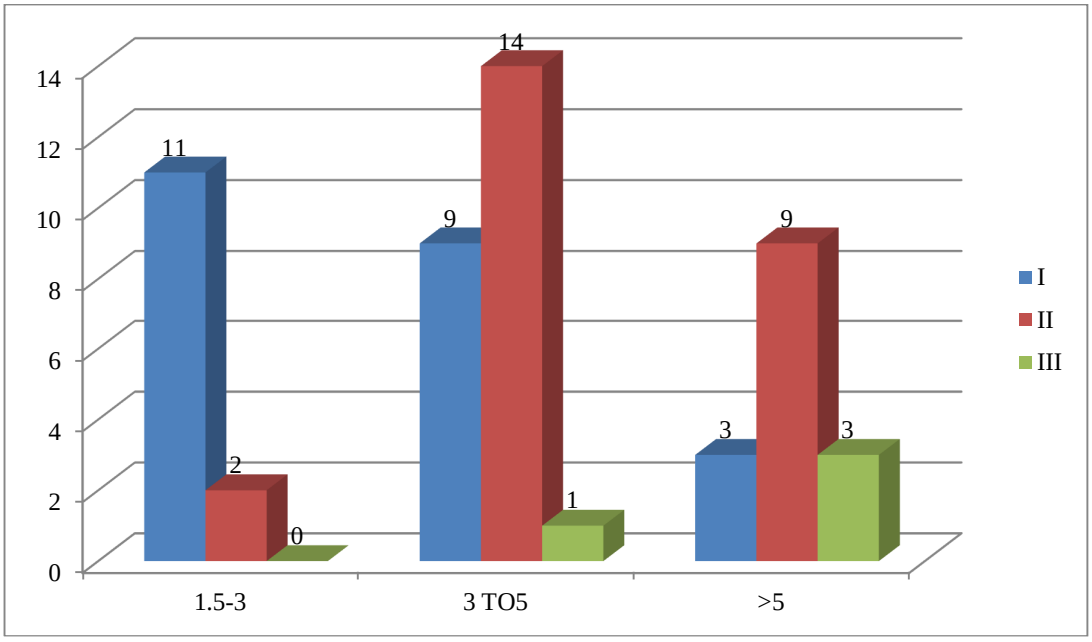
SIZE WISE DISTRIBUTION OF UROTHELIAL CARCINOMA:

Chart 6: showing size wise distribution of urothelial carcinoma:



The mean tumor size of high grade and low grade urothelial carcinoma was 3-5cm. In this study, as the size of tumor increased, the frequency with which the muscle invasion occurs also increased. Thus there exists a significant statistical correlation between tumor size & invasiveness (P value=0.002) but no correlation was observed between size and the grade of the tumor (P value=0.08).

Chart 7: showing comparison of tumor size with stage of the tumor:



Most stage I tumors fall in 1.5-3cm size, stage II tumors were in the 3-5cm range and stage III tumors were more than 5cm in size. Thus, large size of the tumor correlates with higher stage. There was no significant correlation between size and stage of the tumor (P=0.712).

CORRELATION OF URINE CYTOLOGY WITH HISTOPATHOLOGICAL GRADE:

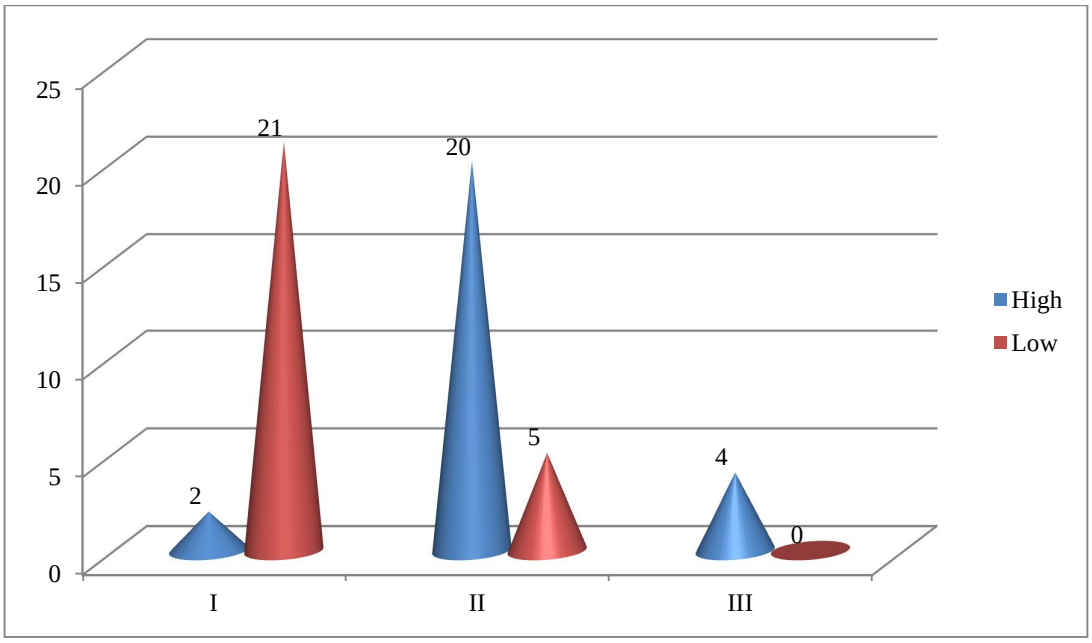
Table 5: showing correlation of urine cytology with histopathological grade:

MICROSCOPY	HIGH		LOW		TOTAL
	INVASIVENESS		INVASIVENESS		
	MI	NMI	MI	NMI	
	Count	Count	Count	Count	Count
ACELLULAR	0	0	0	2	2
ACUTE INFLAMMATORY PATHOLOGY	3	0	0	0	3
DESCRIPTIVE	0	1	0	2	3
NO ATYPICAL CELLS	2	0	0	4	6
POSITIVE FOR MALIGNANCY	19	1	4	12	36
SUGGESTIVE OF MALIGNANCY	0	0	1	0	1
SUSPICIOUS OF MALIGNANCY	0	0	0	1	1
TOTAL	24	2	5	21	52

Out of 26 high grade urothelial carcinoma, 20 cases showed urine cytology smears positive for malignancy and among 26 low grade urothelial carcinoma, 16 cases were positive for malignancy. Thus, most high grade urothelial carcinoma shed their malignant cells in urine and it can be identified in cytology smears.

CORRELATION OF TUMOR GRADE WITH STAGE:

Chart 8: showing correlation of tumor grade with stage:



Pearson chi square=28.696, P=0.001

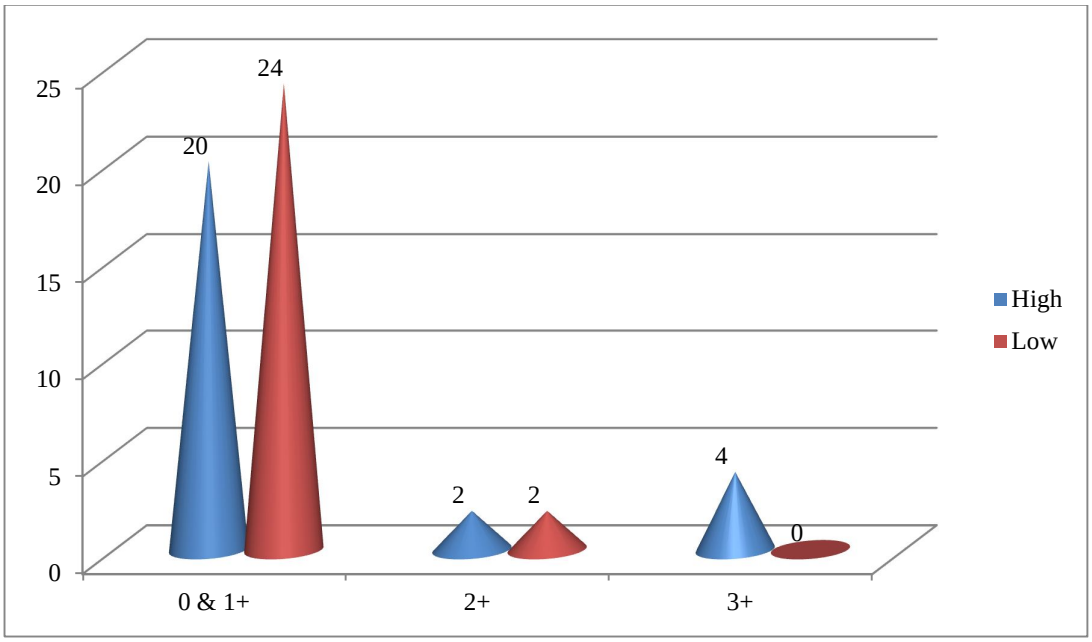
From this chart, most low grade carcinomas were stage I and among 26 high grade urothelial carcinoma, 20 were stage II tumor, 4 were stage III tumor and 2 were stage I tumor. There was a statistical correlation between tumor stage and grade.

RESULTS OF IMMUNOHISTOCHEMICAL STUDIES:

IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU:

The immunohistochemical expression of HER 2 NEU was evaluated by ASCO scoring system and it was scaled from 0-3+ score.

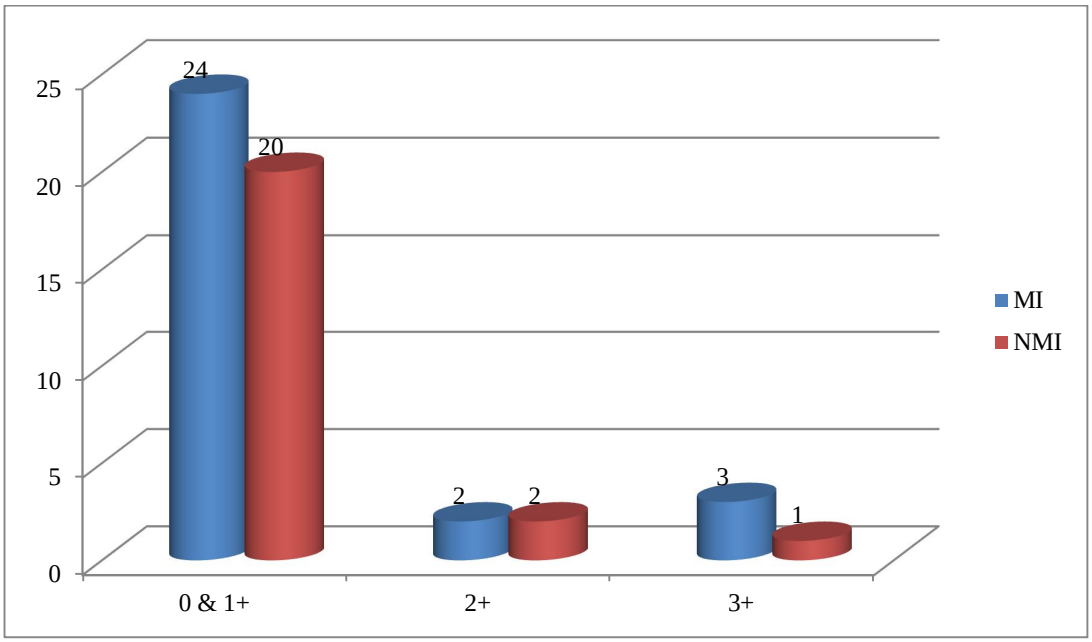
Chart 9: showing correlation between HER2 NEU expression and grade of the tumor:



Pearson chi square=5.909, P=0.05

Among 26 high grade urothelial carcinoma, 20 (77%) were negative, 2 (8%) showed equivocal staining and remaining 4 (15%) showed strong 3+ membranous positivity. Out of 26 low grade urothelial carcinoma, 24 (92%) were negative and 2 (8%) showed equivocal staining. There was a significant statistical correlation between HER 2 NEU expression and grade of the tumor.

Chart 10: showing correlation of HER2 NEU expression with invasiveness:



Pearson chi square=0.680, P=0.712

Among 29 invasive tumors, 24 (83%) were negative, 2 (7%) showed equivocal staining and 3 (10%) cases showed strong 3+ membranous positivity. Among 23 non invasive tumors, 20 (87%) were negative, 2 (9%) showed equivocal staining and 1 (4%) showed strong 3+ membranous positivity. There was no significant statistical correlation between HER 2 NEU expression and invasiveness of the tumor.

Table 6: Showing immunohistochemical expression of HER 2 NEU in urothelial carcinoma:

HER 2 NEU	GRADE				TOTAL
	HIGH		LOW		
	INVASIVENESS		INVASIVENESS		
	MI	NMI	MI	NMI	
	Count	Count	Count	Count	
1+	19	1	5	19	44
2+	2	0	0	2	4
3+	3	1	0	0	4
Total	24	2	5	21	52

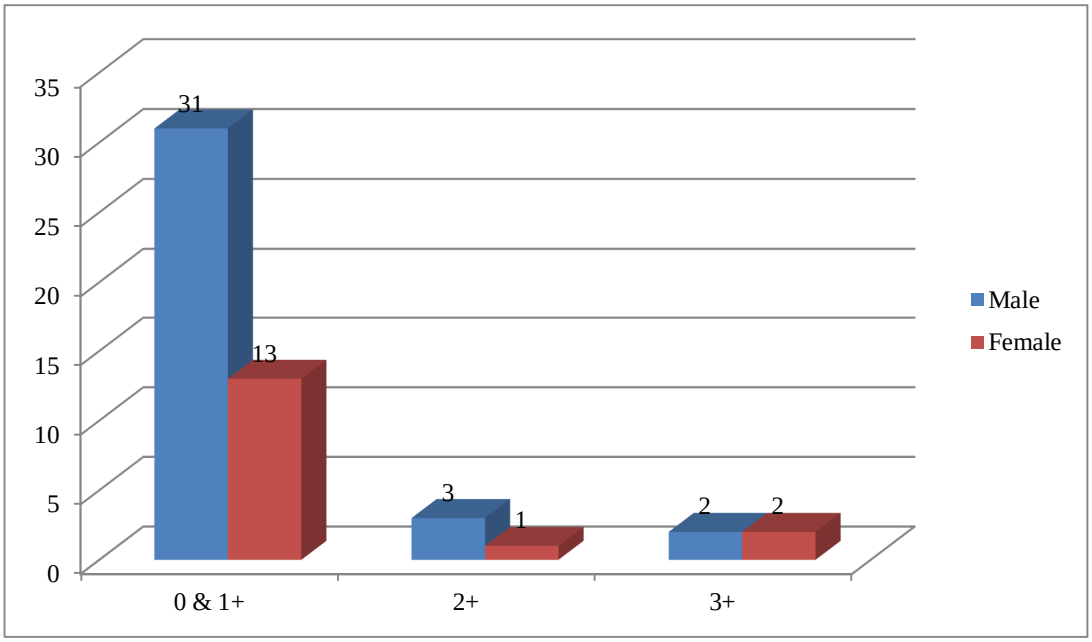
Among 26 high grade urothelial carcinoma, 4 high grade urothelial carcinomas showed 3+ membranous positivity (3 muscle invasive and 1 non muscle invasive). 2 cases showed equivocal positivity which needs further HER 2 NEU demonstration by FISH. Remaining 20 high grade urothelial carcinomas were negative for HER 2 NEU staining.

Among 26 low grade urothelial carcinoma, 2 cases showed equivocal positivity and remaining 24 cases were negative for HER 2 NEU staining.

The inference from this study was that most low grade urothelial carcinomas were negative for HER 2 NEU staining and HER 2 NEU staining among high grade carcinomas were determined by various other factors which

needs further research in the future. HER 2 NEU positive high grade carcinoma patients can be considered for targeted therapy in the future.

Chart 11: showing correlation of HER 2 NEU expression with gender:

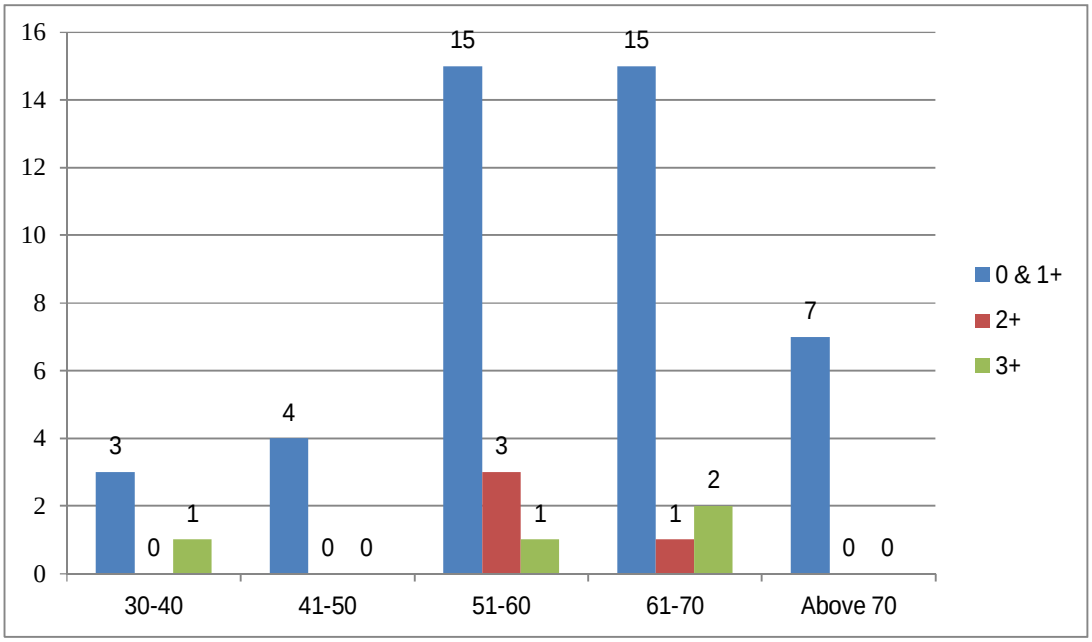


Pearson chi square=0.788, P=0.674

Among 36 male patients, 31 (86%) patients showed negative staining, 3 (8%) patients had equivocal expression and remaining 2 (6%) patients showed strong 3+ membranous positivity. Out of 16 female patients, 13 (81%) showed negative staining, 1 (6%) showed equivocal staining and 2 (13%) patients expressed 3+ membranous positivity. No significant statistical correlation was observed between HER 2 NEU expression and gender.

From this study, it was found that immunohistochemical expression of HER 2 NEU was not affected by gender.

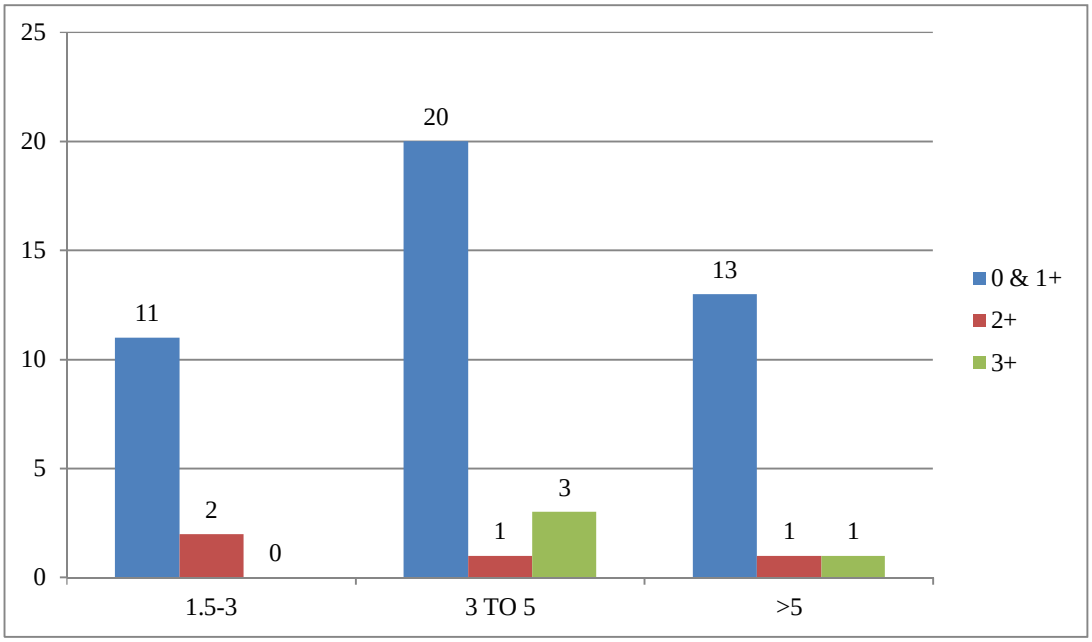
Chart 12: showing correlation of HER 2 NEU expression and age:



Pearson chi square=7.146, P=0.521

In this study, age of the patient was divided into 5 groups starting from 30-40 years, 41-50, 51-60, 61-70 and above 70 years. Among 52 cases, strong 3+ membranous positivity were found in the 61-70 age group (2 cases) and one case each in the 51-60 & 30-40 age group. There was no statistical correlation between HER 2 NEU expression and age of the patient.

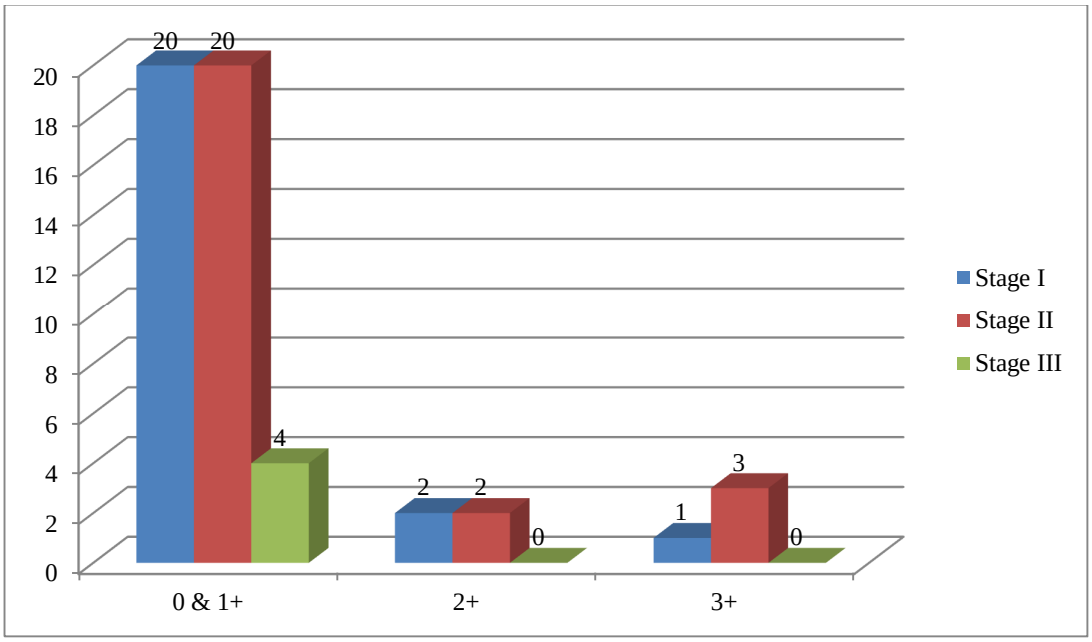
Chart 13: showing correlation of HER 2 NEU expression with tumor size:



Pearson chi square=3.16, P=0.531

From this chart, it was evident that there was variable expression of HER 2 NEU among each size range. Among 52 cases, strong HER 2 NEU expression was found in the 3-5cm range (3 cases) and one case with more than 5cm tumor size showed strong expression. Thus there was no statistical correlation between HER 2 NEU expression and size of the tumor.

Chart 14: showing correlation of HER2 NEU expression with tumor stage:



Pearson chi square=1.776, P=0.777

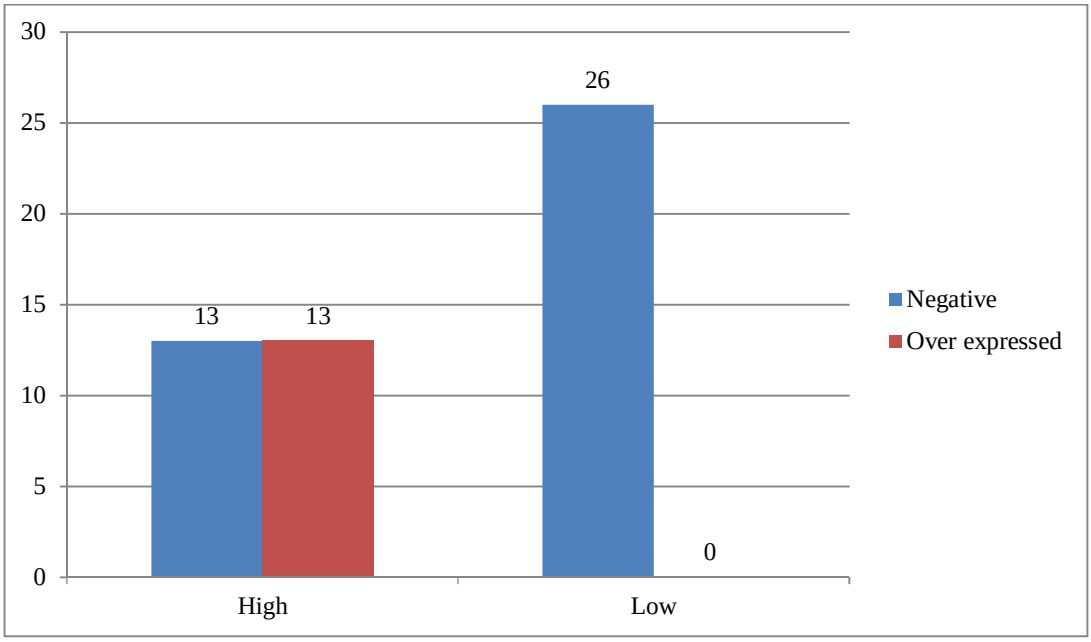
Most stage I and III tumors showed negative and equivocal staining, while stage II tumors exhibited variable expression (20 cases were negative, 2 cases showed equivocal expression, 3 cases showed strong 3+ membranous positivity). There was no statistical correlation between HER 2 NEU expression and stage of the tumor.

From this study, we inferred that HER 2 NEU expression showed significant statistical correlation with grade of the tumor. Several other variables like invasiveness, gender, age, tumor size and stage did not reveal significant statistical correlation with HER 2 NEU expression. Further research has to be implemented in the future to recognize the variables that affect the immunohistochemical expression of HER 2 NEU in bladder carcinoma.

IMMUNOHISTOCHEMICAL EXPRESSION OF P53:

The nuclear positivity in more than 10% of the cells were considered as over expression and expression in less than 10% of the cells were considered negative. In this study, the following criterias have been adhered while evaluating the results.

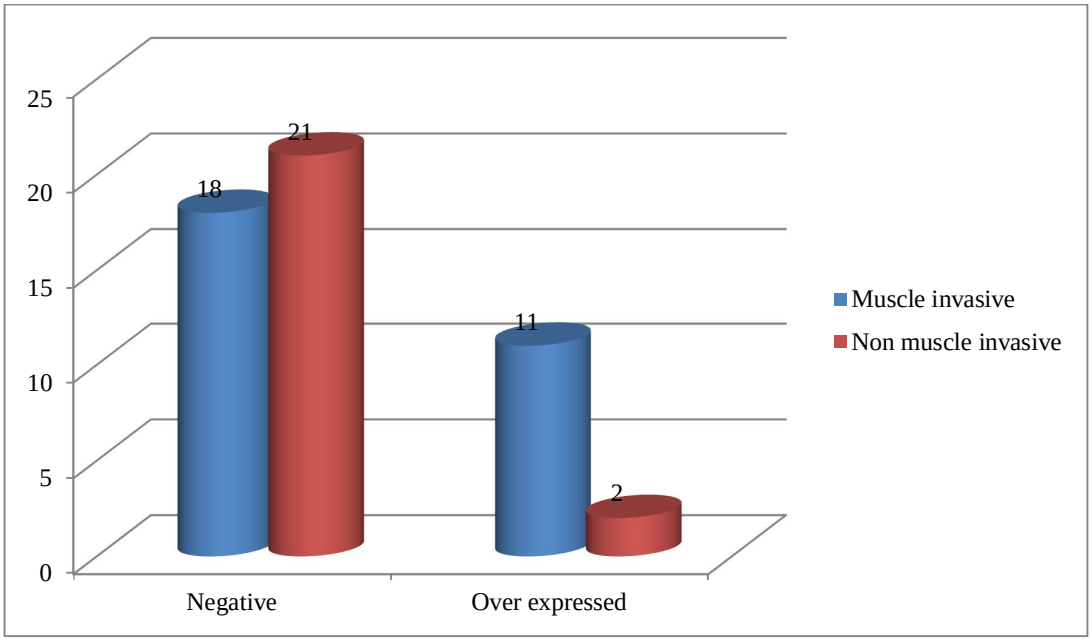
Chart 15: showing P53 expression among high & low grade urothelial carcinoma



Pearson chi square=17.333, P<0.001

Among 26 high grade urothelial carcinoma, 13 (50%) were negative and remaining 13 (50%) showed over expression of P53. Out of 26 low grade urothelial carcinoma, all 26 cases (100%) showed negative P53 staining. Thus P53 expression showed significant statistical correlation with tumor grade.

Chart 16: showing correlation of P53 expression with invasiveness of the tumor



Pearson chi square=5.847, P=0.016

Among 29 invasive tumors, 18 (62%) were negative, 11 (38%) showed over expression of P53. Among 23 non invasive tumors, 21 (91%) were negative, 2 (9%) showed over expression of P53. It was inferred that there exists a significant statistical correlation between P53 over expression and the invasive nature of the tumor.

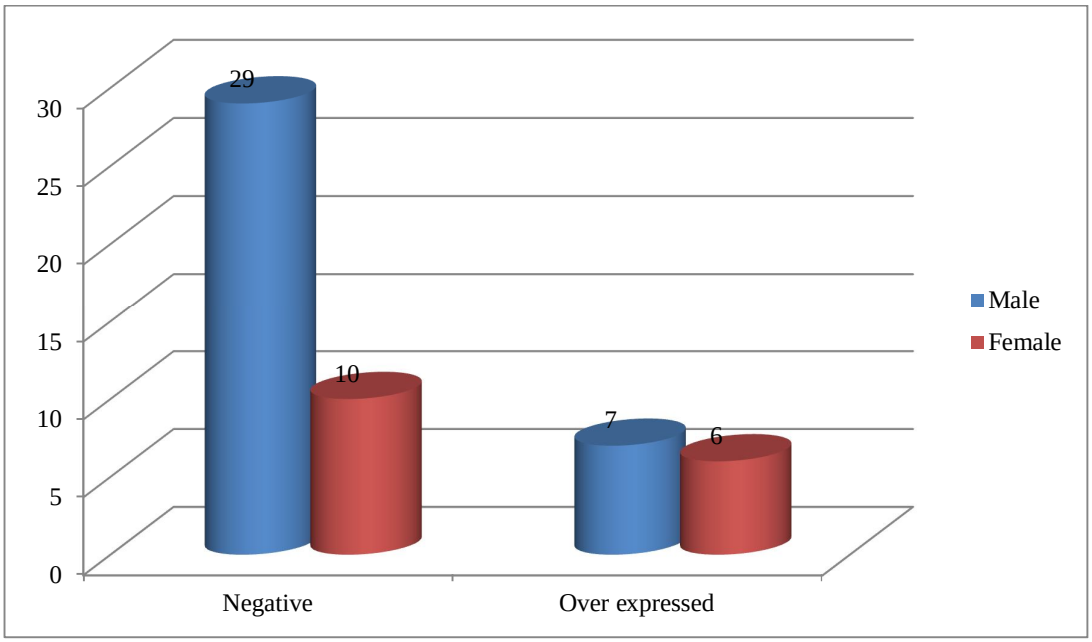
Table 7: showing immunohistochemical expression of P53 in urothelial carcinoma:

P53	GRADE				TOTAL
	HIGH INVASIVENESS		LOW INVASIVENESS		
	MI	NMI	MI	NMI	
	Count	Count	Count	Count	
NEGATIVE	13	0	5	21	39
OVER EXPRESSED	11	2	0	0	13
TOTAL	24	2	5	21	52

Among 26 high grade urothelial carcinoma,13 high grade urothelial carcinoma showed negative staining for P53 (all 13 were muscle invasive) and remaining 13 high grade urothelial carcinoma exhibited P53 over expression (11 were muscle invasive and 2 were non muscle invasive).

All 26 low grade urothelial carcinomas were negative for P53 over expression. The inference from this study was that all low grade urothelial carcinomas were negative for P53 staining and 50% of high grade urothelial carcinoma over expressed P53 protein and it can be considered as one of the prognostic factor.

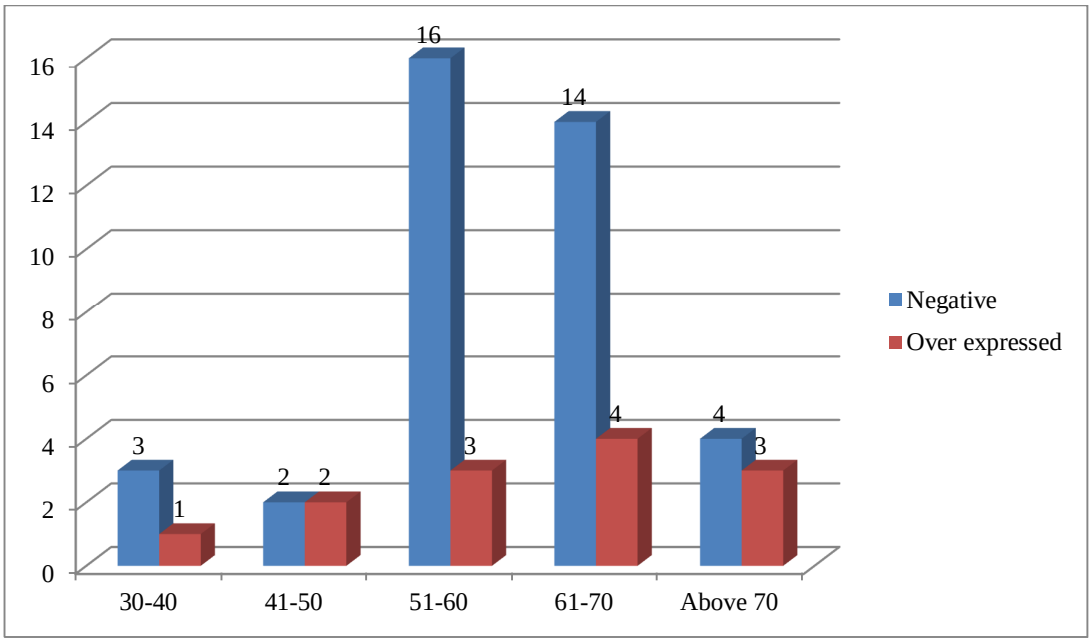
Chart 17: showing correlation between P53 expression with gender



Pearson chi square=1.926, P=0.165

Among 36 male patients, 29 patients (81%) showed negative staining and remaining 7 (19%) patients showed over expression of P53 staining. Out of 16 female patients, 10 (63%) showed negative staining and 6 (37%) showed over expression of P53 protein. Thus P53 staining does not show significant statistical correlation and its expression was independent of gender.

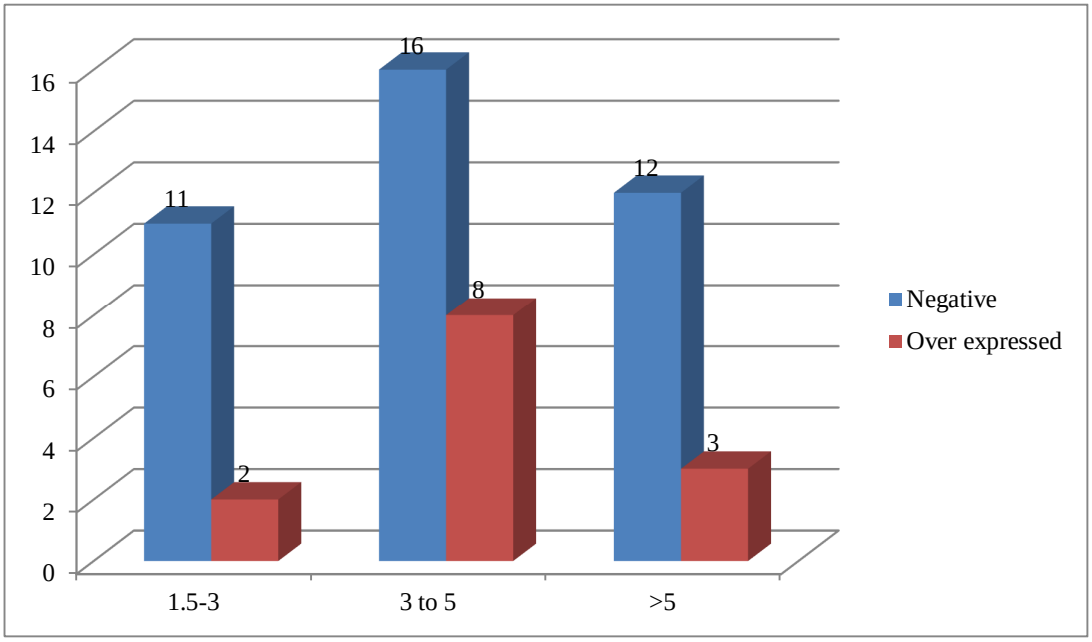
Chart 18: showing correlation of P53 expression with age:



Pearson chi square=3.458, P=0.519

This chart depicts variable expression of P53 among each range of age group and there was not much statistical correlation between P53 expression and was independent of age.

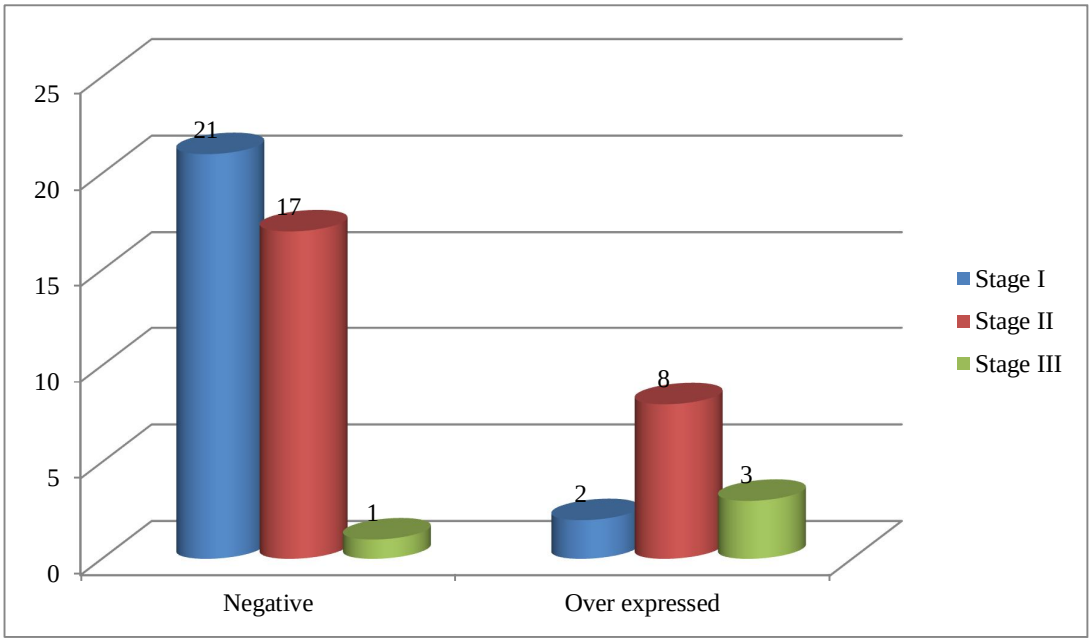
Chart 19: showing correlation between P53 expression with tumor size



Pearson chi square=1.73, P=0.421

P53 showed variable staining pattern in each range of the tumor size and tumor more than 5cm in size showed over expression of P53. There was no significant statistical correlation between tumor size and P53 over expression.

Chart 20: showing correlation between P53 expression with stage:



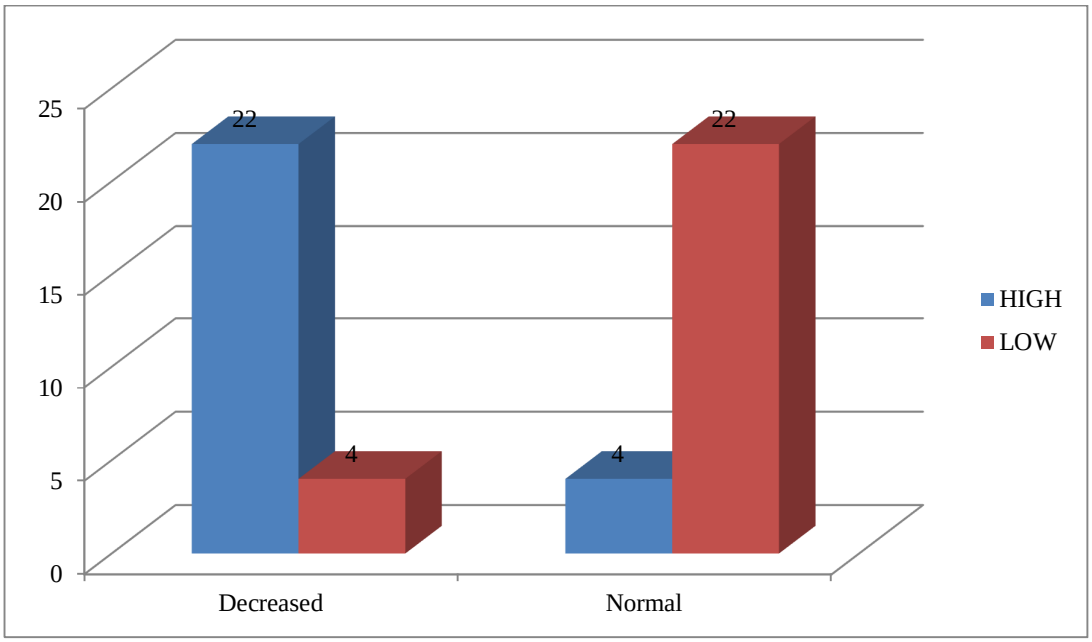
Pearson chi square=9.248, P=0.010

Most stage I and stage II tumors were negative for P53 staining, while stage III tumors over expressed P53 protein. Thus P53 over expression was statistically associated with stage of the tumor and might affect the outcome of the patient.

IMMUNOHISTOCHEMICAL EXPRESSION OF P63:

The cut off for decreased expression of P63 was nuclear positivity in less than 90% of the tumor cells and expression in more than 90% of the cells were considered normal and these criterias have been followed through out the P63 evaluation.

Chart 21: showing P63 expression among high & low grade urothelial carcinoma

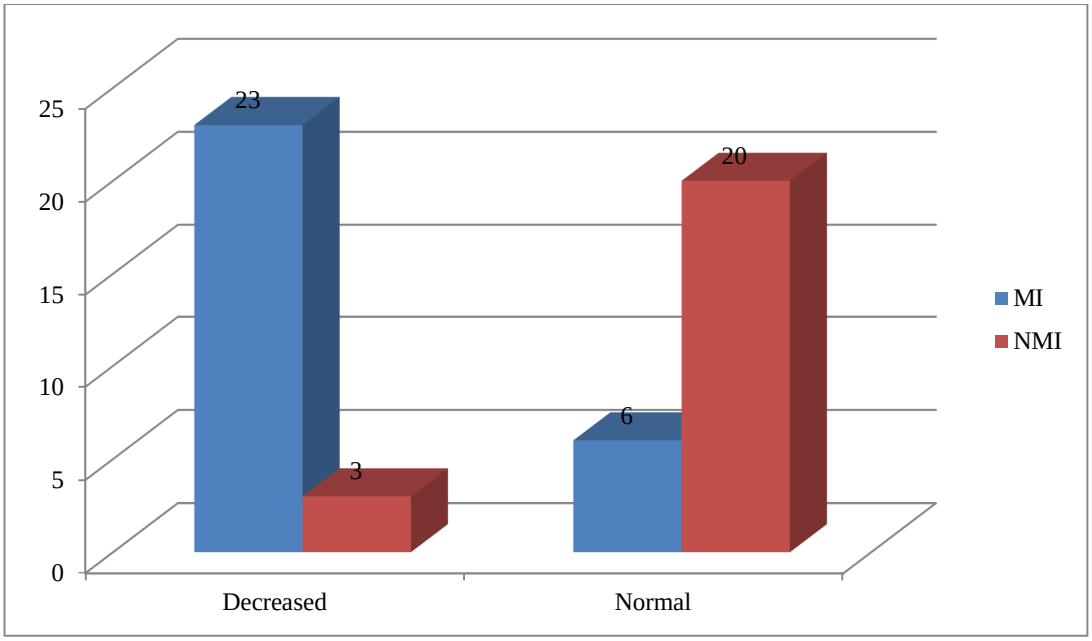


Pearson chi square=24.923, P<0.001

Among 26 high grade urothelial carcinoma, 22 cases (85%) showed decreased P63 expression and 4 cases (15%) retained their normal P63 expression. Out of 26 low grade urothelial carcinoma, only 4 cases (15%) showed decreased P63 expression and remaining 22 cases (85%) showed normal P63 expression. It was evident that there exists a significant statistical correlation between P63

expression and grade of the tumor. Thus most high grade urothelial carcinomas lost their normal P63 expression while low grade urothelial carcinomas retained their normal P63 expression.

Chart 22: showing distribution of P63 expression among muscle & non muscle invasive tumors



Pearson chi square=22.531, P<0.001

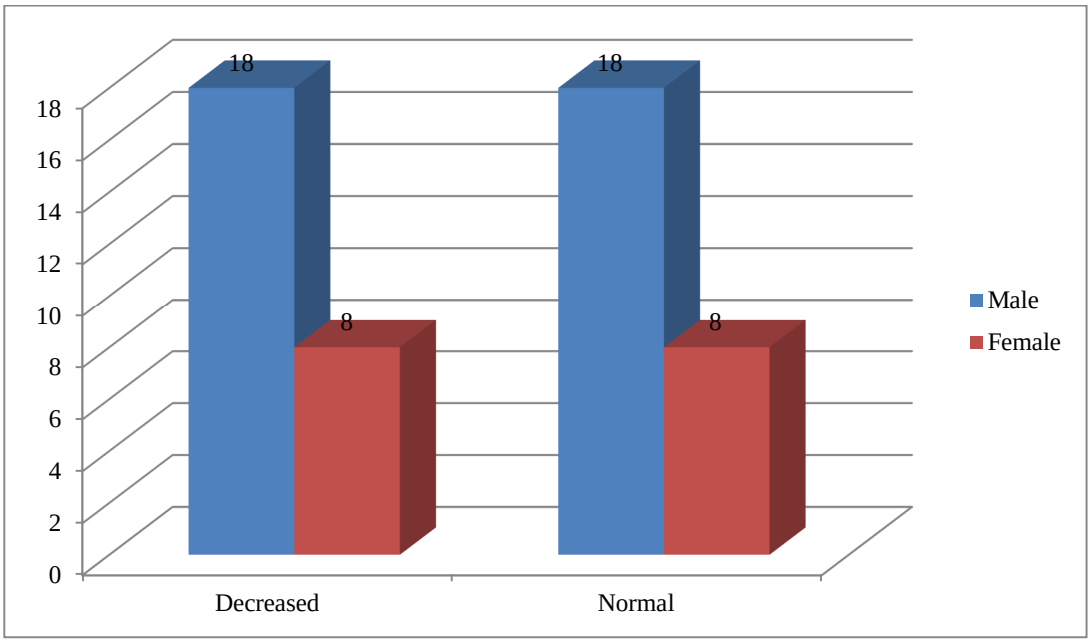
Out of 29 invasive tumors, 23 (79%) muscle invasive tumors showed decreased P63 expression and remaining 6 cases (21%) retained their normal expression. Among 23 non muscle invasive tumors, 20 cases (87%) retained normal expression and remaining 3 cases (13%) showed decreased expression. P value was significant and shows a strong correlation between P63 expression and invasiveness of the tumor. Thus invasiveness of the tumor correlated directly with decreased P63 expression and can be considered as one of the prognostic factor.

Table 8: showing P63 expression among urothelial carcinoma

P63	GRADE				TOTAL
	HIGH INVASIVENESS		LOW INVASIVENESS		
	MI	NMI	MI	NMI	
	Count	Count	Count	Count	
DECREASED	21	1	2	2	26
NORMAL	3	1	3	19	26
TOTAL	24	2	5	21	52

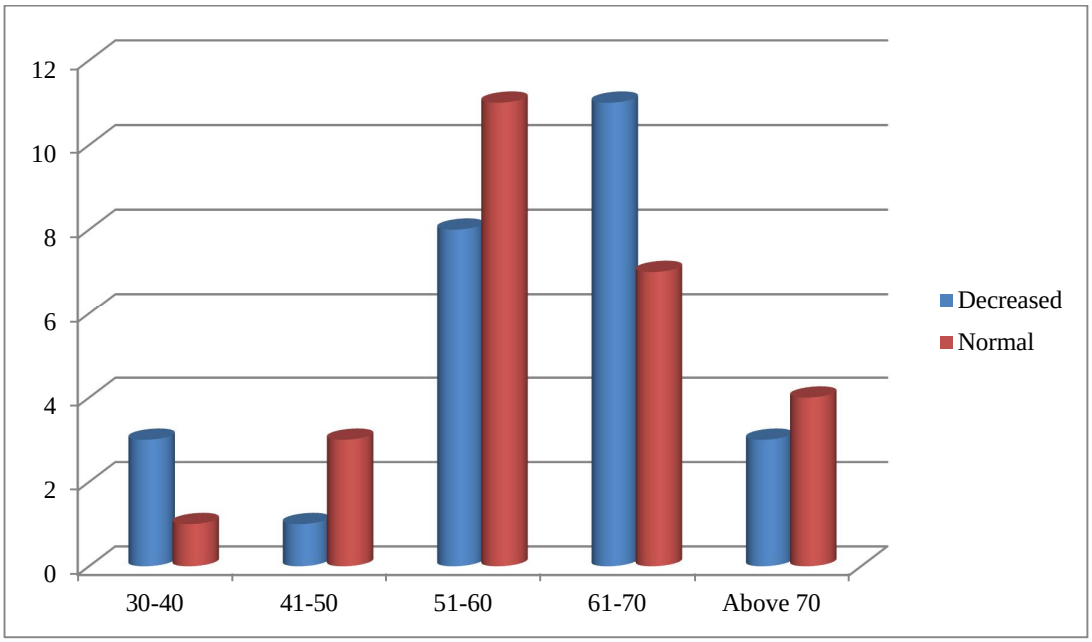
Among 26 high grade urothelial carcinoma, 22 cases showed decreased expression (21 were muscle invasive & 1 non muscle invasive) and remaining 4 cases retained their normal expression (3 muscle invasive & 1 non muscle invasive). Out of 26 low grade urothelial carcinoma, 4 cases showed decreased expression (2 muscle invasive & 2 non muscle invasive) and remaining 22 cases retained their normal expression (3 muscle invasive & 19 non muscle invasive). Thus most high grade carcinoma had decreased P63 expression and low grade urothelial carcinoma retained their P63 expression.

Chart 23: showing correlation of P63 expression with gender



Among 36 male patients, 50% (18 cases) showed decreased P63 expression and 50% (18 cases) retained normal P63 expression. Out of 16 female patients, 50% (8 cases) showed decreased P63 expression and 50% (8 cases) retained their normal expression. Thus P63 expression was independent of gender. There was no significant statistical correlation with P63 expression and gender (P=1).

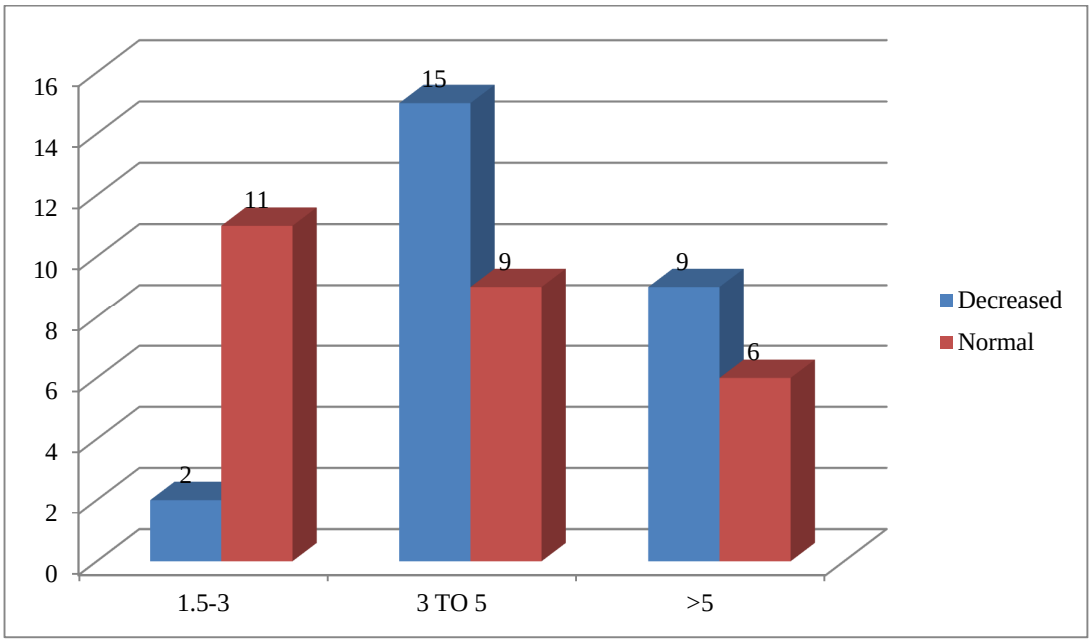
Chart 24: showing correlation of P63 expression with age



Pearson chi square=3.505, P=0.462

From this chart, highest number of cases demonstrating decreased P63 expression falls in the 61-70 age group and there was variable expression of P63 in each age group. It was evident that there was no significant correlation between decreased expression of P63 and age.

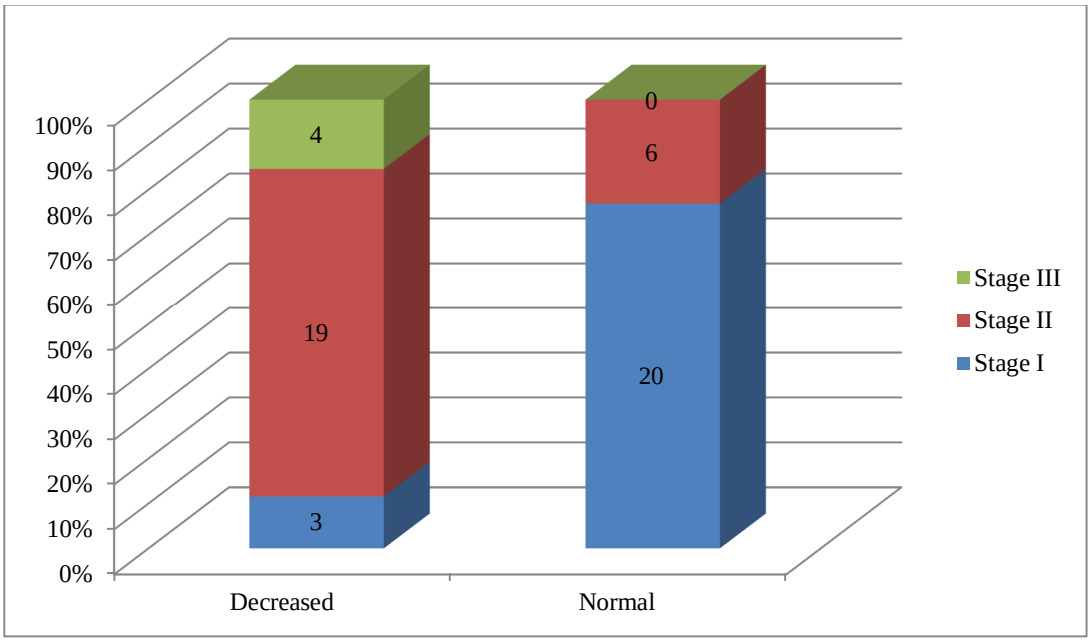
Chart 25: showing correlation of P63 expression with tumor size



Pearson chi square=8.33, P=0.016

Majority of cases showing decreased P63 expression falls in the size range of 3-5cm and statistical correlation was observed between decreased expression of P63 and tumor size.

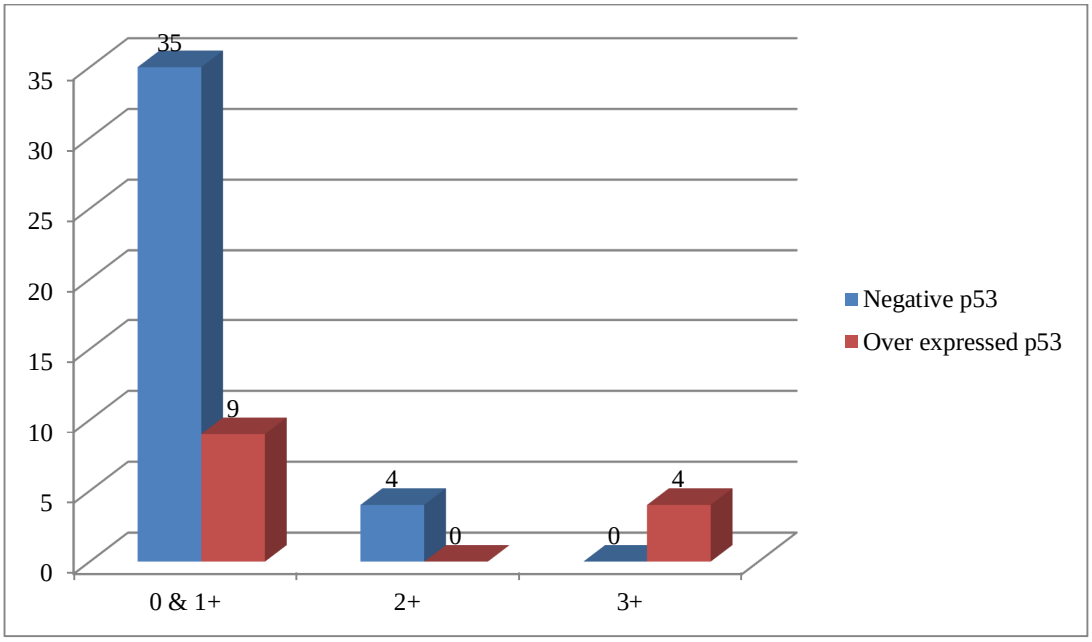
Chart 26: showing correlation of P63 expression with stage



Pearson chi square=23.325, P=0.001

In this study, most stage I tumors retained their normal P63 expression while majority of stage II & III tumors showed decreased P63 expression. It was clear that P63 expression showed statistical correlation with tumor stage and it can be considered as one of the prognostic factor in the future.

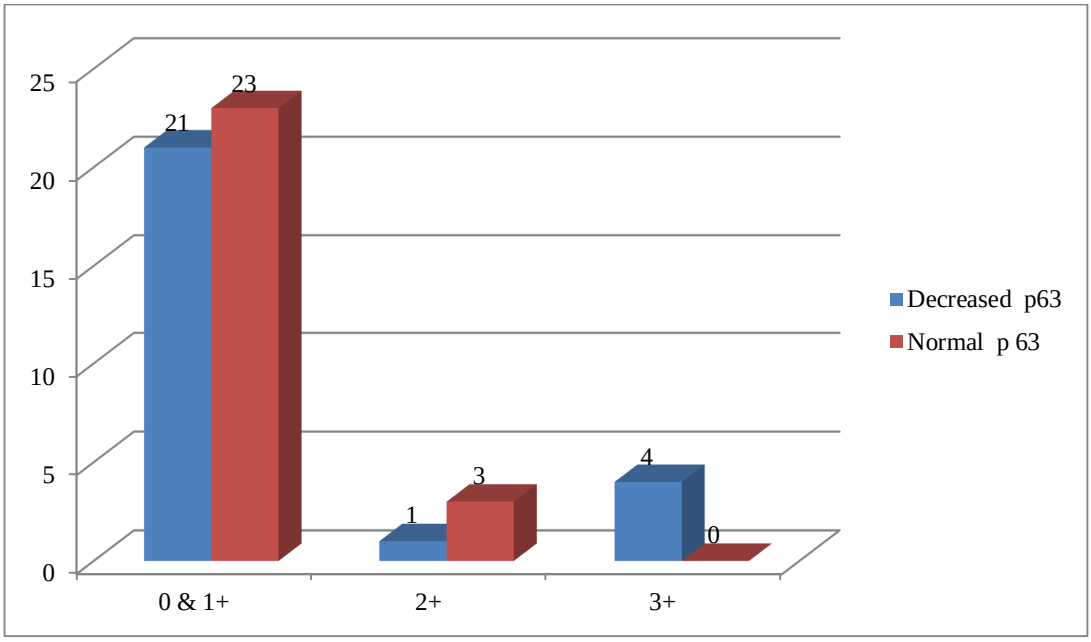
Chart 27: showing correlation between HER 2 NEU expression & P53 expression



Pearson chi square=13.818, P=0.001

Among total 52 cases, 44 cases showed negative HER 2 NEU staining, 4 cases showed equivocal staining pattern and remaining 4 cases showed strong 3+ membranous staining. These 4 cases which showed strong 3+ membranous HER 2 NEU positivity revealed over expression of P53. P value was significant and showed statistical correlation between HER 2 NEU expression and P53 expression.

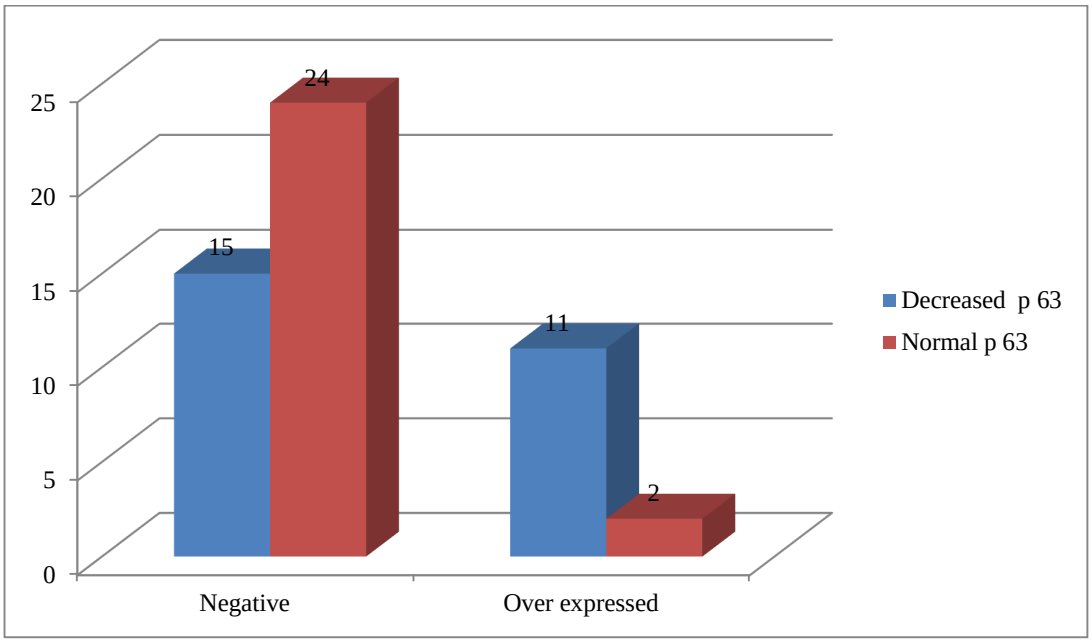
Chart 28: showing correlation between HER 2 NEU expression & P63 expression



Pearson chi square=6.683, P=0.035

Among total 52 cases, 44 cases showed negative HER 2 NEU staining, 4 cases showed equivocal staining pattern and remaining 4 cases showed strong 3+ membranous staining. These 4 cases which showed strong 3+ membranous HER 2 NEU positivity revealed decreased expression of P63. Thus, HER 2 NEU positivity showed statistical correlation with decreased expression of P63 protein and both HER 2 NEU positivity with decreased expression of P63 can be considered as the prognostic factors in urothelial carcinoma.

Chart 29: showing correlation between P53 expression &P63 expression



Pearson chi square=8.308, P=0.004

Among 52 cases, 39 cases showed negative P53 staining and 13 cases showed over expression of P53 protein. Out of 13 cases which over expressed P53 protein, 11 cases showed decreased P63 expression and 2 cases retained normal P63 expression. It was evident that there was a statistical correlation between P53 over expression & decreased expression of P63 protein and they could be considered as prognostic factors in the future.

Colour Plates

Fig.8:BX 1923/17 – ANTERIOR PELVIC EXENTERATION-CUT SURFACE

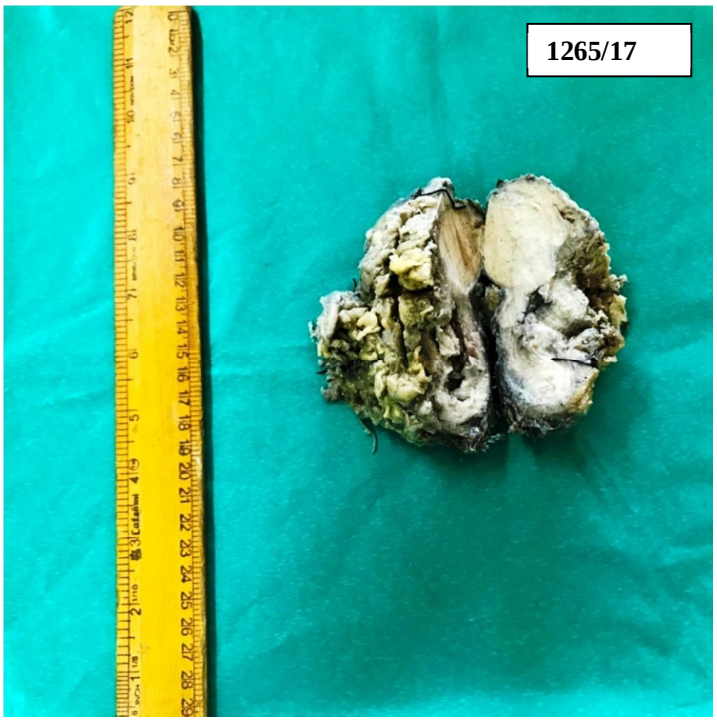


Fig.9: BX 1265/17 – RADICAL CYSTECTOMY-CUT SURFACE

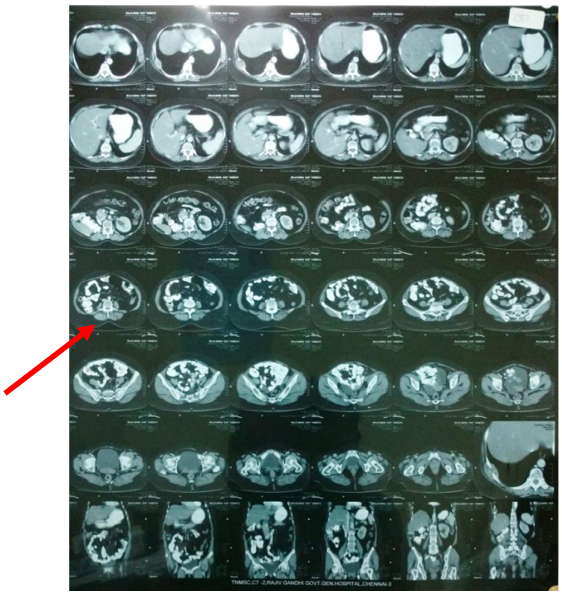


Fig 10: CT showing two soft tissue density lesion arising from urinary bladder

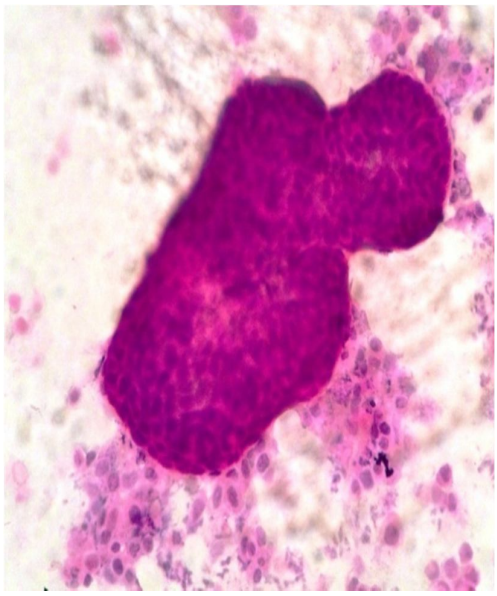


Fig 11: C-1265/17 showing papillaroid configuration of malignant urothelial cells- 400X (H&E)

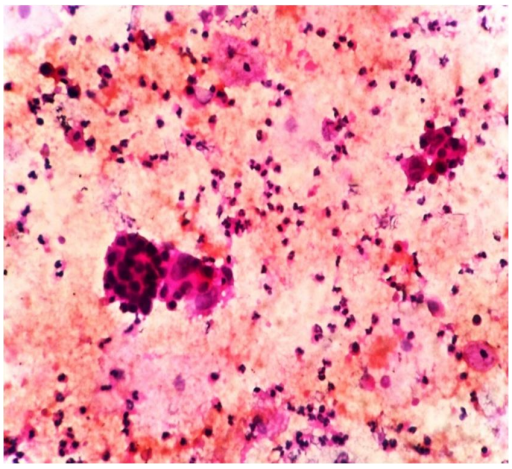


Fig 12: C-1589/17 showing clusters of malignant urothelial cells in an inflammatory background-100X (H&E)

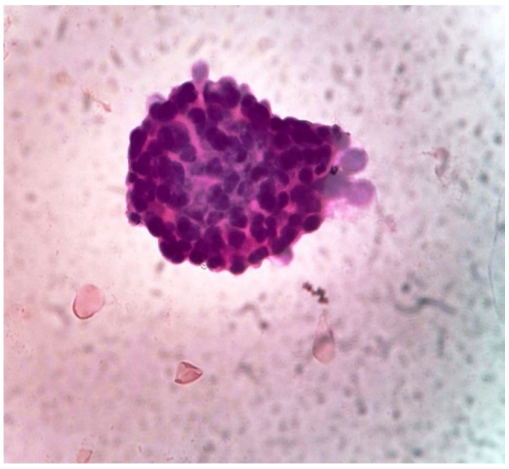


Fig 13: C-2904/17 showing malignant urothelial cluster-100X (H&E)

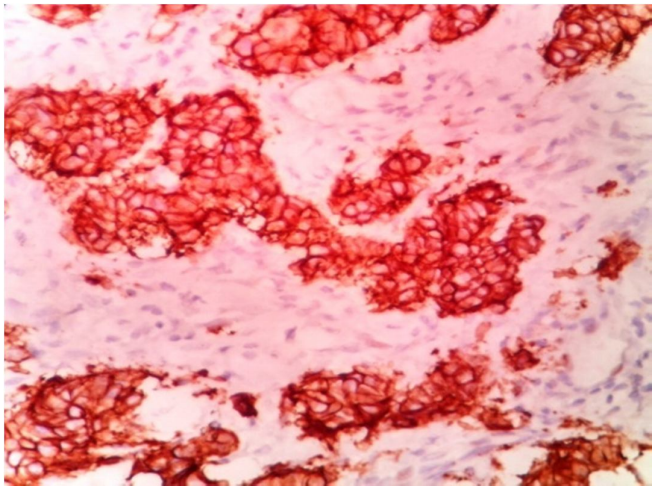


Fig 14: showing strong 3+ membranous HER 2 NEU staining in breast carcinoma (positive control) - 400X

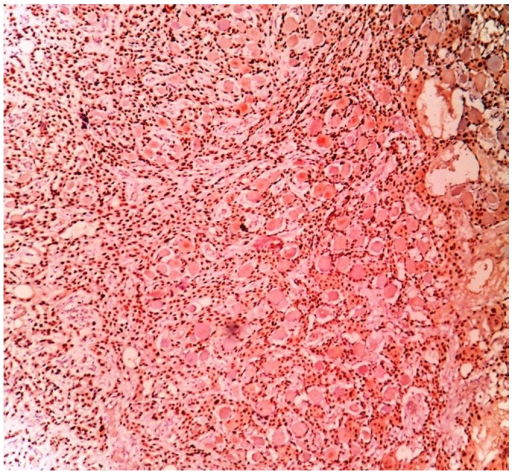


Fig 15: showing nuclear staining of P53 in colon carcinoma(positive control)- 100X

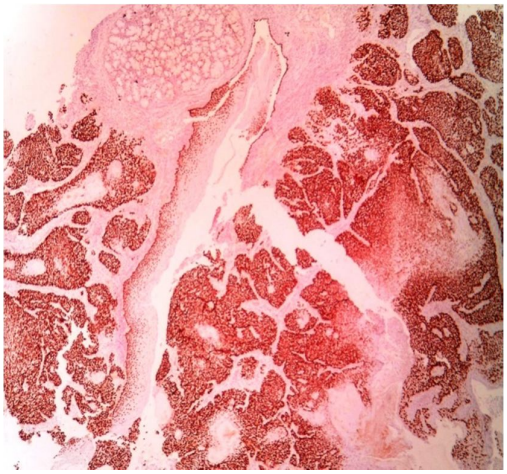


Fig 16: showing nuclear staining of P63 in squamous cell carcinoma of oral cavity(positive control)- 100X

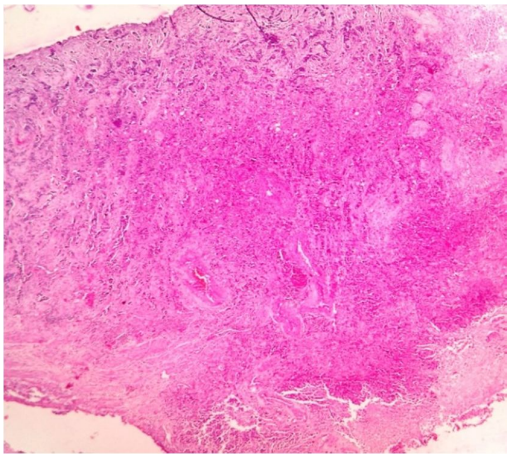


Fig 17:BX 318/17 High grade MI urothelial carcinoma (H&E)-100X

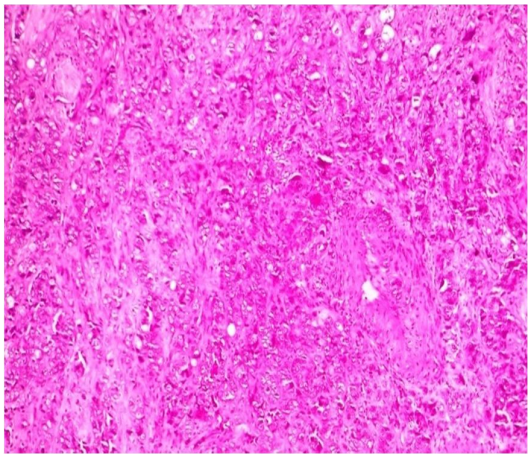


Fig 18: BX 318/17 High grade MI urothelial carcinoma (H&E)-400X

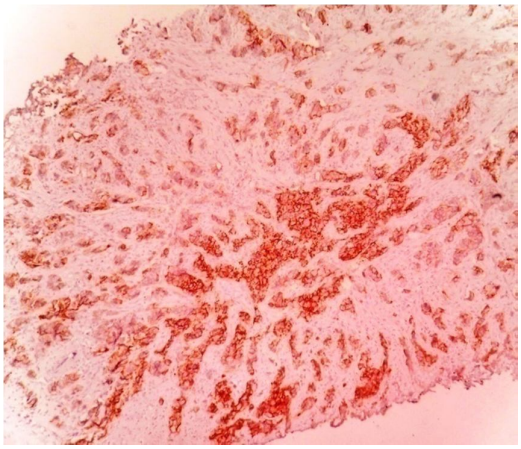


Fig 19: BX 318/17 strong 3+ membranous staining of HER 2-100X

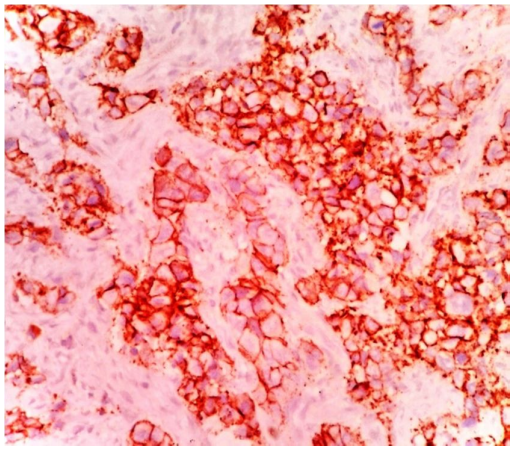


Fig 20: BX 318/17 strong 3+ membranous staining of HER 2-400X

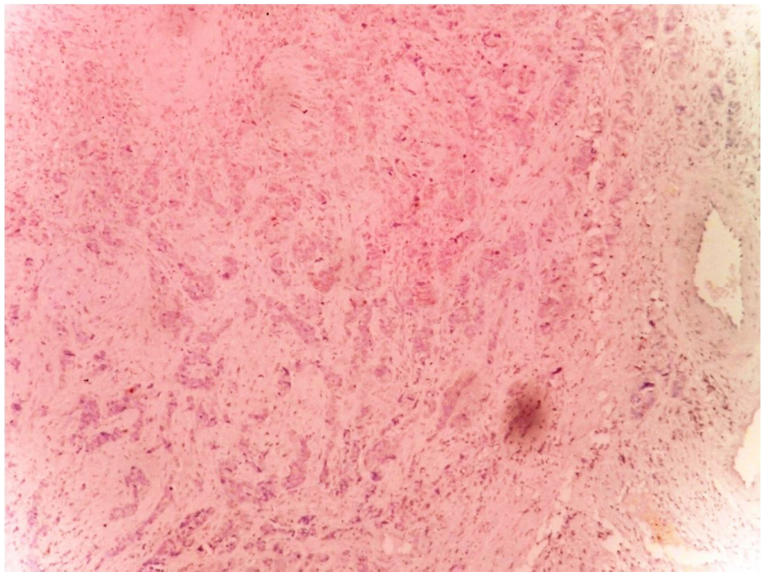


Fig 21: BX 318/17 Negative P63 staining in malignant cells-400X

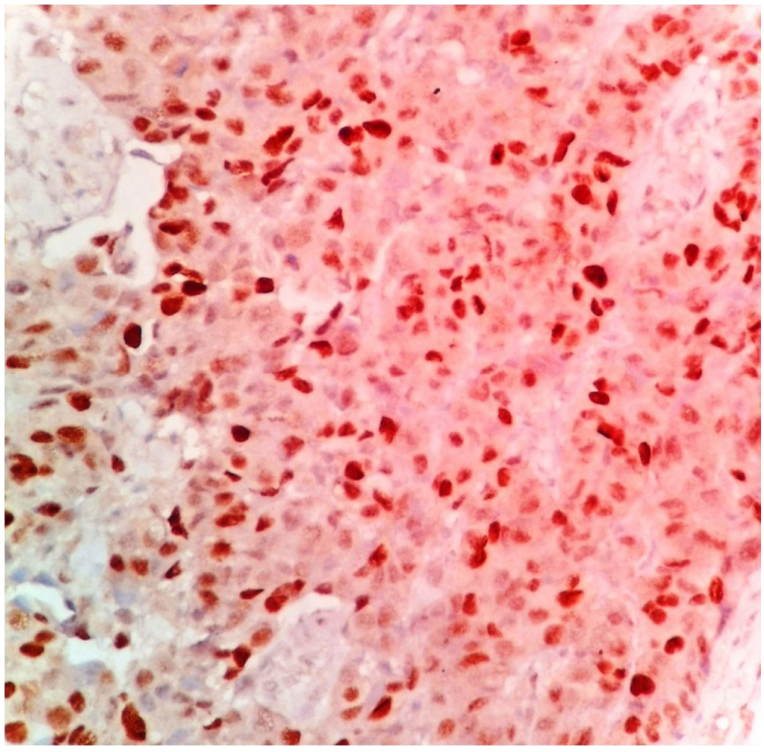


Fig 22: BX 318/17 Nuclear positivity of P53 protein in malignant cells-400X

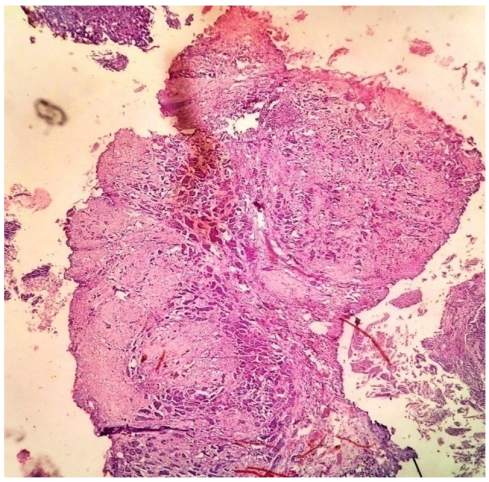


Fig 23: BX 1589/17 High grade MI urothelial carcinoma (H&E) -40X

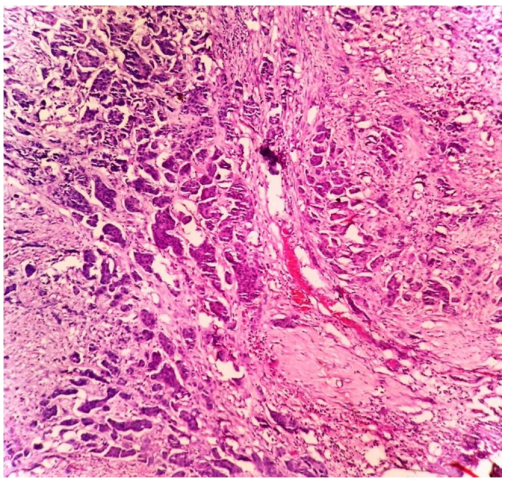


Fig 24: BX 1589/17 High grade MI urothelial carcinoma (H&E) - 400X

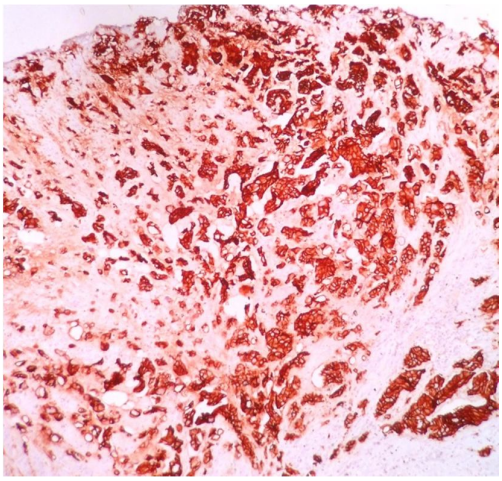


Fig 25: BX 1589/17 strong 3+ membranous staining of HER2-40X

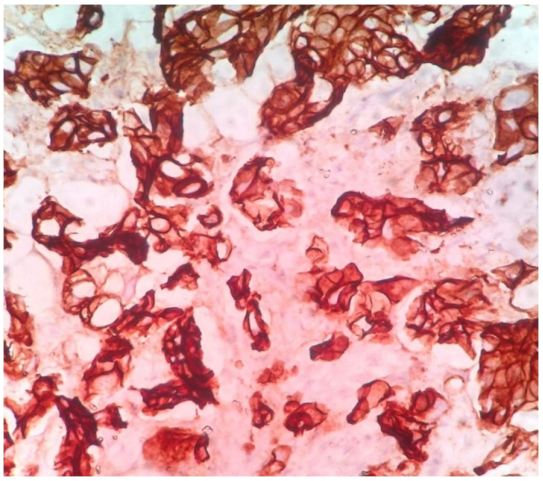


Fig 26: BX 1589/17 strong 3+ membranous staining of HER2- 400X

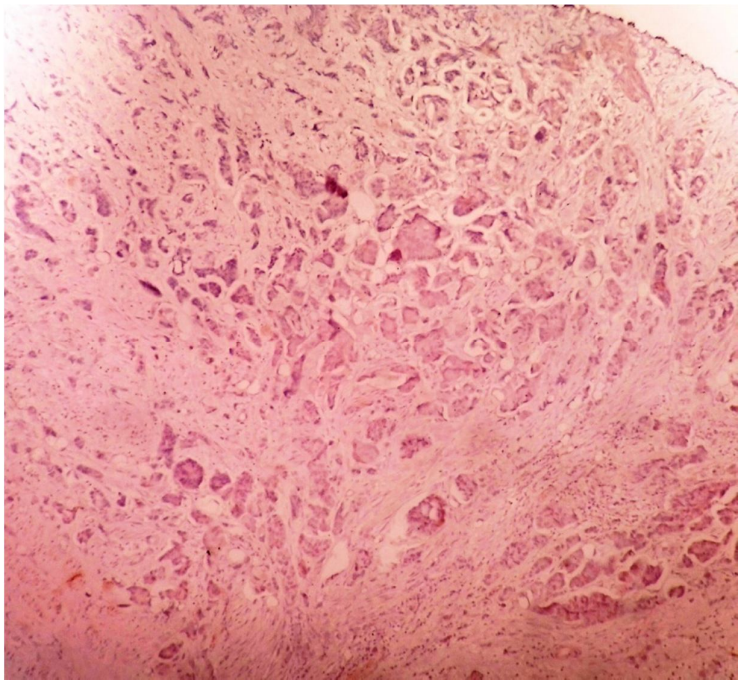


Fig 27: BX 1589/17 Negative P63 staining-100X

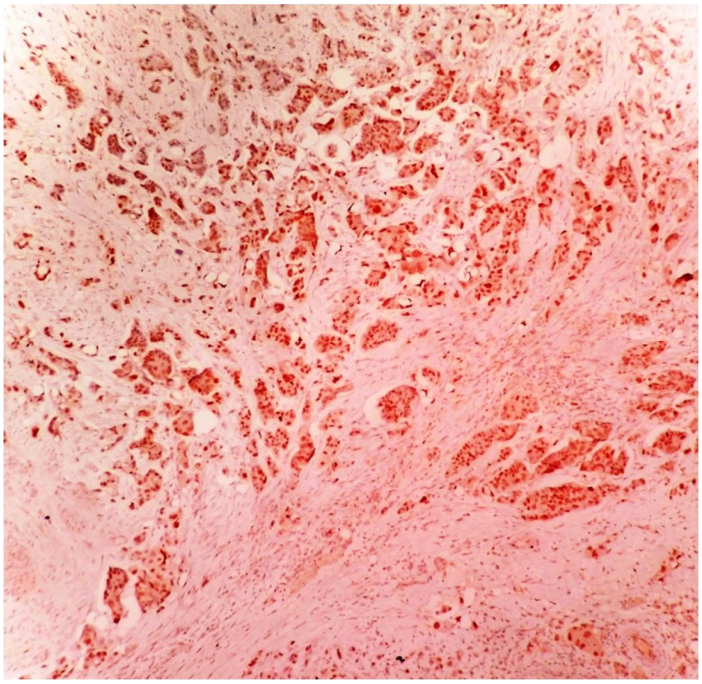


Fig 28: BX 1589/17 Nuclear positivity of P53 protein-100X

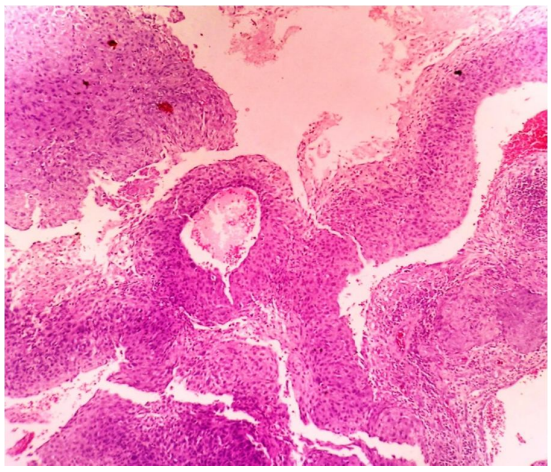


Fig 29: BX 3097/17 High grade NMI urothelial carcinoma (H&E)-100X

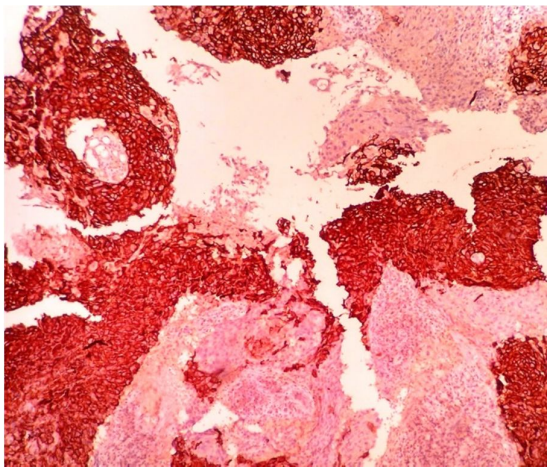


Fig 30: BX 3097/17 strong 3+ membranous staining of HER2-100X

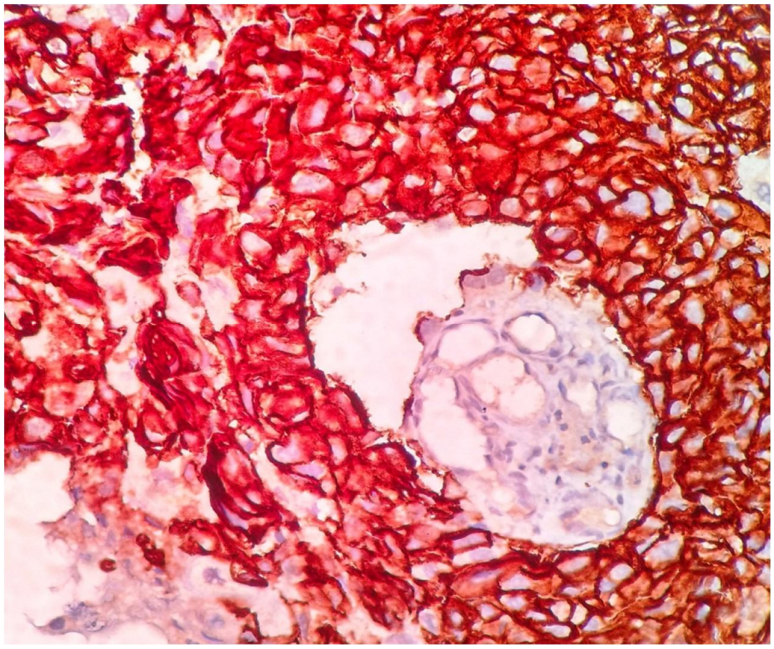


Fig 31: BX 3097/17 strong 3+ membranous staining of HER2-400X

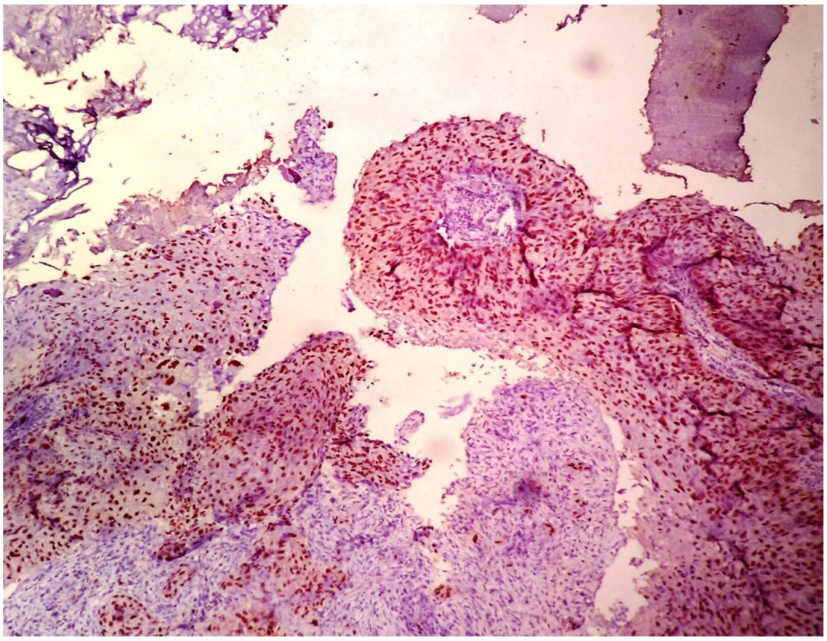


Fig 32: BX 3097/17 Nuclear positivity of P53 protein-100X

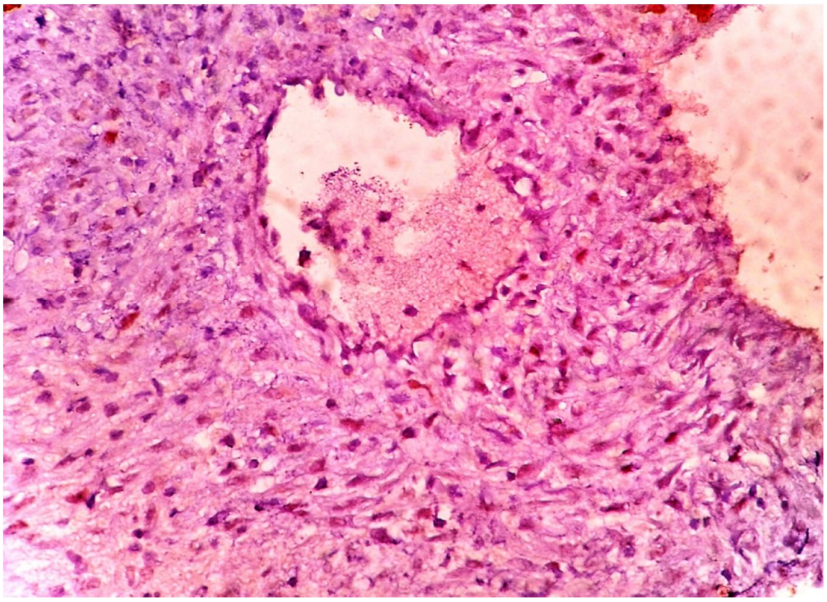


Fig 33: BX 3097/17 Negative P63 staining-100X

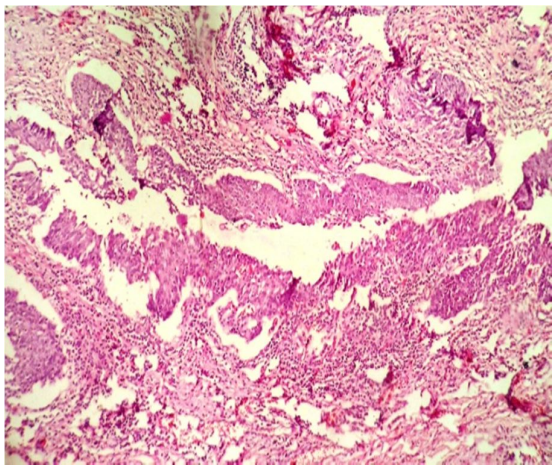


Fig 34: BX 1265/17 Low grade NMI urothelial carcinoma (H&E)-100X

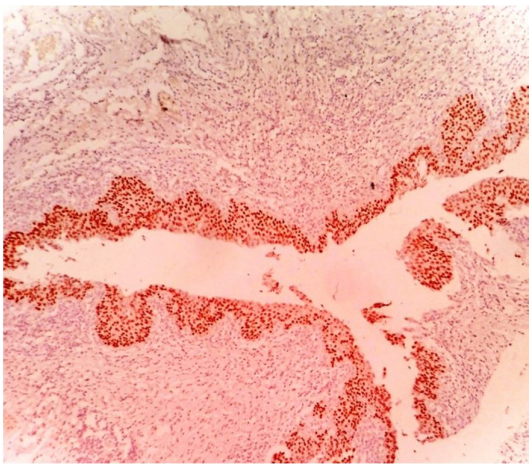


Fig 35: BX 1265/17 strong nuclear positivity of P63 protein-100X

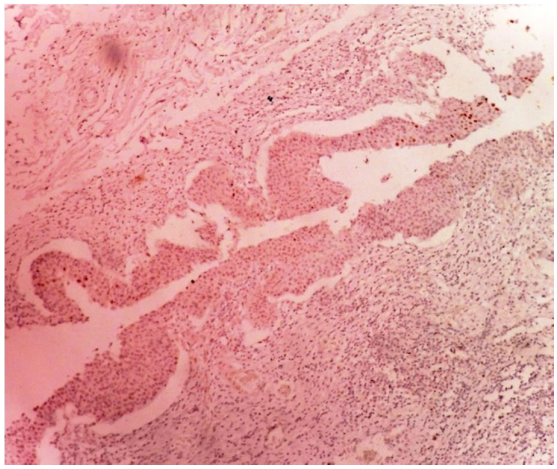


Fig 36: BX 1265/17 Negative HER2 staining

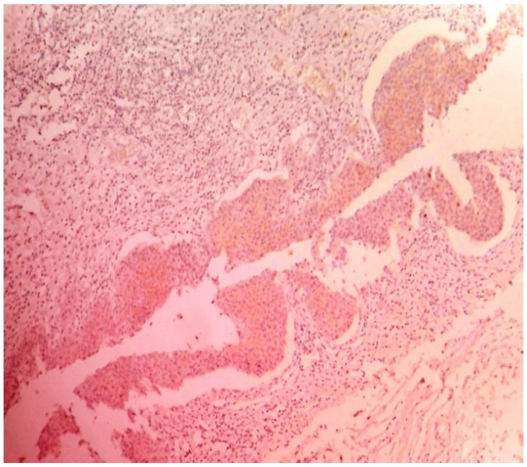


Fig 37: BX 1265/17 Negative P53 protein

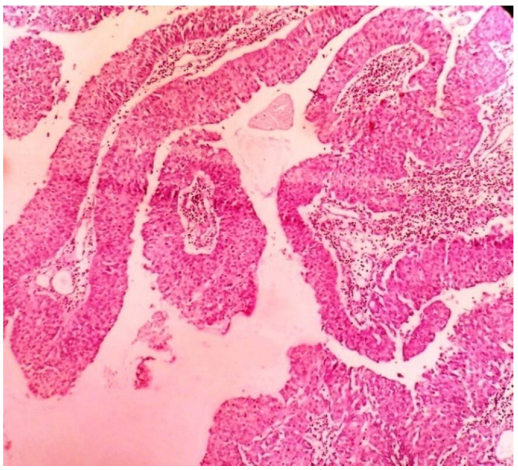


Fig 38: BX 1577/16 Low grade NMI urothelial carcinoma (H&E)-100X

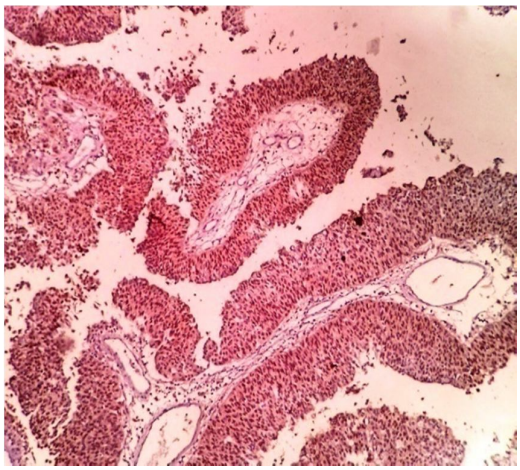


Fig 39: BX 1577/16 strong nuclear staining of P63 protein-100X

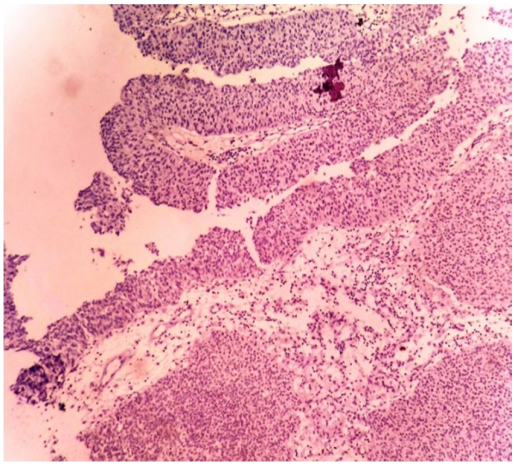


Fig 40: BX 1577/16 Negative HER2 staining

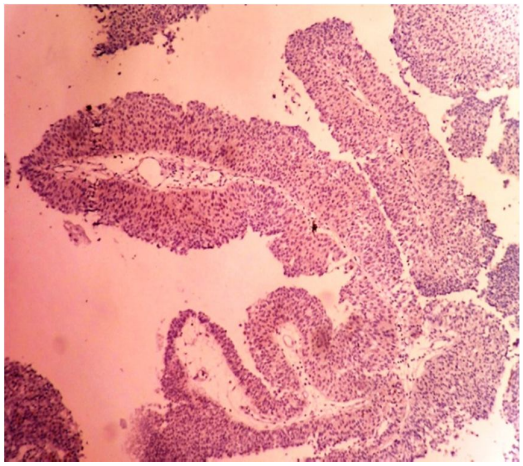


Fig 41: BX 1577/16 Negative P53 protein staining

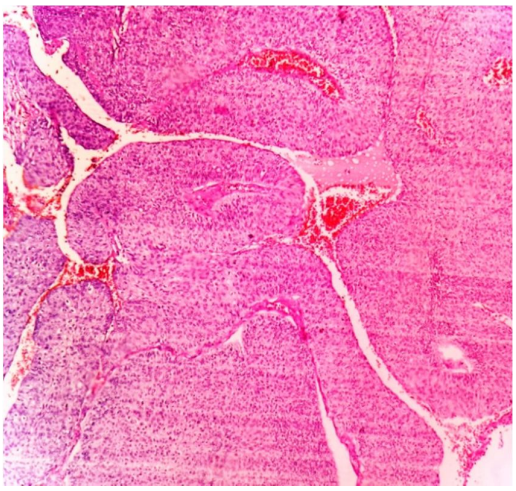


Fig 42: BX 2572/17 Low grade MI urothelial carcinoma (H&E)-100X

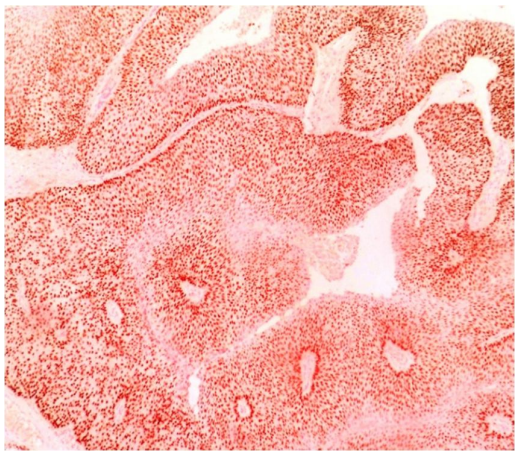


Fig 43: BX 2572/17 Nuclear positivity of P63 protein-100X

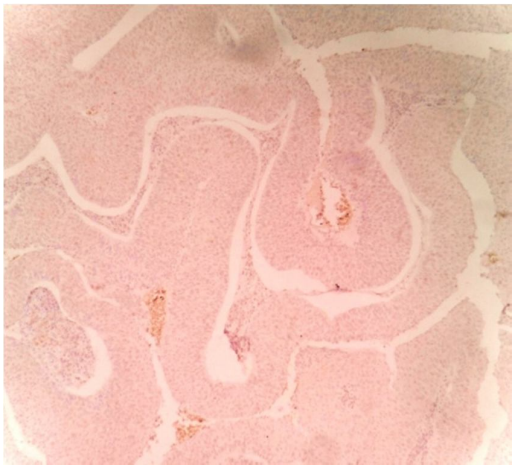


Fig 44: BX 2572/17 Negative HER2 staining-100X

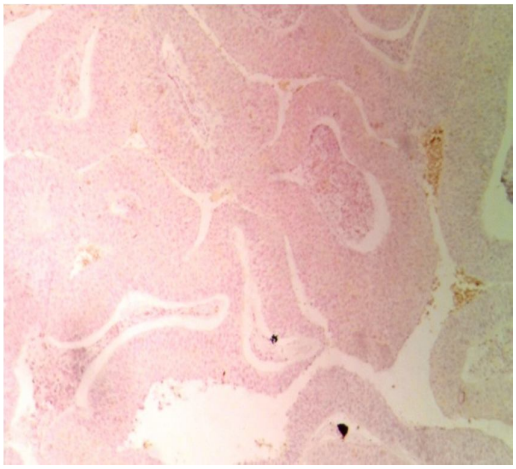


Fig 45: BX 2572/17 Negative P53 protein staining-100X

Discussion

DISCUSSION

The most common malignant tumor of the urinary system is transitional cell carcinoma and accounts for more than 90% of all bladder tumors[24]. As per the Indian cancer registry data, it is the ninth most common cancer and is three times more common in men than in women[26]. Various prognostic factors have influenced the outcome of patient with urothelial carcinoma. In this study, we evaluated the immunohistochemical expression of HER 2 NEU, P53 and P63 in muscle invasive and non muscle invasive bladder tumors and correlated their expression with various clinico pathological variables like age, gender, tumor size, site, grade, stage and invasiveness of the tumor.

We received 123 bladder specimens and 84 were reported as urothelial carcinoma. The incidence of urothelial carcinoma in our Institute was 68% (84 cases). Among 68% of urothelial carcinoma, low grade urothelial carcinomas constituted 45% (38 cases) and high grade urothelial carcinomas accounted for 55% of cases (46 cases). Based on the invasive nature, further stratification of high and low grade urothelial carcinoma was made. Out of 46 high grade urothelial carcinoma (55%), 39 cases (85%) were muscle invasive and 7 cases (15%) were non muscle invasive. Among 38 low grade urothelial carcinoma (45%), 9 (24%) were muscle invasive and remaining 29 (76%) were non muscle invasive.

Several studies showed variable incidence of muscle invasive and non muscle invasive tumors. According to Gupta et al. 561 bladder cancer patients

were included in the study and only 26% of the patients had muscle invasive disease at the time of presentation [148]. In most studies non muscle invasive tumors were frequently encountered than muscle invasive tumors (18%).

Our Institute being a tertiary referral center, high grade urothelial carcinomas outnumbered the low grade urothelial carcinomas. Among high grade urothelial carcinoma, muscle invasive tumors were most frequently encountered than non muscle invasive tumors and most low grade urothelial carcinoma were non muscle invasive tumors.

Out of 84 urothelial carcinomas, equal proportion of high grade (26 cases) and low grade (26 cases) carcinomas were selected for easy comparison. Among 26 high grade urothelial carcinomas, 24 (92%) were muscle invasive and 2 (8%) were non muscle invasive tumors. Out of 26 low grade urothelial carcinomas, 5 (19%) were muscle invasive and 21 (81%) were non muscle invasive. The discrepancy in selecting muscle invasive and non muscle invasive tumor occurred due to the high incidence of muscle invasive tumor among high grade and most low grade urothelial carcinoma were non muscle invasive.

TYPE OF SPECIMEN RECEIVED:

Among 123 bladder specimens, 86 were TURBT specimens (70%) and remaining 37 (30%) were radical cystectomy specimens. In the study group consisting of 52 urothelial carcinoma (100%), 39 (75%) were TURBT specimens and 13 (25%) were radical cystectomy specimens which included palliative

cystectomy and anterior pelvic exenteration specimen. In our Institute, TURBT was performed more frequently than cystectomy.

AGE WISE INCIDENCE OF UROTHELIAL CARCINOMA:

In this study, the peak incidence of urothelial carcinoma was above 50 years of age. The mean age group for high grade urothelial carcinoma was 61-70 years and for low grade urothelial carcinoma was 51-60 years. The incidence of bladder carcinoma is low among young age group and as age advances tumor grade is higher. In a study conducted by Humphrey et al. the incidence of urothelial carcinoma is seen in patients over 50 years of age [(2)] and the incidence correlates with our study.

In patients more than 50 years of age, the invasive nature of tumor is more in high grade urothelial carcinoma than in low grade urothelial carcinoma.The tumor grade correlates directly with age and as age advances, the tumor grade is higher [(149)].

In this study, the highest number of stage I tumor (9 cases) occurred in 51-60 age group. The maximum number of stage II tumor (10cases) was found in 51-60 years age group. The stage III tumor was found above 60 years of age with the exception of one case falling in 41-50 age group. It is inferred that stage III tumors were found in higher age group. Thus, with respect to advanced age, the tumor grade and stage showed correlation and affects the outcome of the patients[(149)].

GENDER WISE DISTRIBUTION OF UROTHELIAL CARCINOMA:

Among 52 urothelial carcinoma, 69% (36 cases) were males and females constituted 31% (16 cases). In this study, the male:female ratio for bladder carcinoma was 2:1.According to Mungan et al [(150)], the incidence of urothelial cancer is 3 to 4 times more common in males than in females and it is in par with the present study.

Out of 26 high grade urothelial carcinoma, 65% (17 cases) were male and 35% (9 cases) were females. Among 26 low grade urothelial carcinoma, males constituted 73% (19 cases) and females accounted for 27% (7 cases).

Among high and low grade urothelial carcinoma, males outnumbered females and it is evident that urothelial carcinoma is more common in males than in females.

SITE AND SIZE WISE DISTRIBUTION OF CASES:

In the present series, the most common site of urothelial carcinoma was lateral wall (32 cases) followed by posterolateral wall (13 cases). Other locations like anterior wall, anterolateral wall, base and trigone were less commonly involved. Urothelial carcinoma predominantly involves the lateral and the posterior bladder wall close to the ureteral orifice [(151)] and it is concordant with our study.

In this study, the mean size of the tumor was in the range of 3-5cm for both high (14 cases) and low grade urothelial carcinoma (10 cases). The mean size of

muscle invasive tumor was 3-5cm (15 cases) and for non muscle invasive carcinoma was 1.5-3 cm (11 cases).

According to Heney et al, tumors larger than 5cm showed progression to muscle invasive tumors and this is found in 35% of patients with superficial tumors compared to patients with small bladder tumors [152].

It is inferred that the grade of tumor is independent of its size whereas the invasiveness of the tumor depends on the tumor size.

CORRELATING THE SIZE AND STAGE OF THE TUMOR:

11 cases of stage I tumor have tumor size ranging between 1.5-3cm, 9 cases in the 3-5cm range and 3 cases showed tumor size falling in the range over 5cm.

Most stage II tumors (14 cases) have their range between 3-5cm and most stage III tumors were over 5cm size.

According to Bostwick et al, tumor size influenced the stage of tumor, but not progression to muscle invasive tumors[153]. In the present study, tumor size showed correlation with the invasiveness of the tumor and showed no correlation with stage and grade.

CORRELATING THE URINE CYTOLOGY WITH GRADING:

Of 26 low grade urothelial carcinoma, 16 cases showed positive cytology and in 2 cases smears were suspicious of malignancy and in correlation with histopathological examination all 16 cases turned to be low grade and the 2 cases which were suspicious of malignancy were found to be malignant. Among 26 high grade urothelial carcinoma, 20 cases showed urine cytology smears positive and cytology was not contributory in remaining 6 cases.

According to Layla S Abdullah et al [(154)], the sensitivity of urine cytology is higher in high grade urothelial carcinoma than in low grade urothelial carcinoma.It is evident that percentage of positivity in urine cytology is slightly on the higher range for high grade than low grade urothelial carcinoma because high grade urothelial carcinoma exhibit dyscohesive clusters of malignant cells and thus can be identified in cytology smears.

CORRELATION OF TUMOR GRADE AND STAGE:

In this study, 23 cases (44%) were found to be stage I tumor, 25 (48%) were stage II and 4 cases (8%) were stage III tumor. Of 23 stage I tumors, 2 (9%) were high grade and 21(91%) were low grade urothelial carcinoma. Among 25 stage II tumors, 20 (80%) were high grade and remaining 5 (20%) were low grade carcinoma. 4 cases (100%) of stage III tumors were high grade.

In this study, at the time of presentation, most high grade carcinoma tend to present as stage II & III tumors compared to low grade urothelial carcinoma

which presented as stage I & II tumors. A significant statistical correlation was observed between tumor grade and stage (P=0.001).

IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU, P53 & P63:

Immunohistochemical expression of HER 2 NEU, P53 & P63 was studied in 52 cases of urothelial carcinoma and its expression is compared with clinical variables like gender, age, size of the tumor and pathological variables like histological grade, invasiveness of the tumor, stage of the tumor.

We also compared the expression of HER 2 NEU with P53, HER2 NEU with P63 and P53 with P63. The comparison between immunohistochemical expression of HER 2 NEU, P53 and P63 with various clinico pathological variables is aimed at arriving a relationship between these variables and indirectly aiding in management, risk stratification and outcome of the patient.

IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 NEU:

Among 26 high grade urothelial carcinoma, negative (score 0) to weak membranous positivity (score 1) was expressed in 20 cases (77%), 2 cases (8%) revealed equivocal staining pattern (score 2), 4 cases (15%) expressed strong membranous positivity (score 3). Out of 26 low grade urothelial carcinoma, 24 cases (92%) showed negative to weak membranous positivity, 2 cases (8%) showed equivocal staining pattern and no case showed strong membranous positivity.

According to Bolenz et al , HER 2 NEU was over expressed in 21.7% of high grade urothelial carcinoma and less than 9% of low grade urothelial carcinoma over expressed HER 2 NEU. In a study conducted by Lae et al, HER 2 NEU was over expressed only in 5.1% (strong 3+) of high grade urothelial carcinoma. Several other studies reported variable staining pattern ranging from 21.7% to 80%. [155,156].

Various hypotheses could explain the variable HER 2 NEU staining pattern and can be attributed to various factors like variability in immunohistochemistry assays, the heterogeneity between kits, antibodies, protocols, interpretation of staining pattern. There is no consensus for defining the HER 2 NEU over expression in bladder carcinoma.

In the present series, only score 3+ is considered as over expression and score 2+ should not be considered as over expression without demonstrating HER 2 NEU gene amplification by in situ hybridization. According to Lae et al and Caner et al, cases with score 2+ did not show HER 2 NEU gene amplification.

It is evident that 15% of high grade urothelial carcinoma expressed strong membranous positivity (score 3) whereas 92% of low grade urothelial carcinoma did not express HER 2 NEU.

CORRELATING THE EXPRESSION OF HER 2 NEU WITH THE INVASIVENESS OF THE TUMOR:

Of 29 muscle invasive tumors, 24 cases (83%) had negative to weak membranous positivity, 2 cases (7%) showed equivocal staining pattern and 3 cases (10%) expressed strong membranous positivity.

Among 23 non muscle invasive tumors, 20 cases (87%) exhibited negative to weak staining pattern, 2 cases (9%) showed equivocal staining pattern and 1 case (4%) expressed strong membranous positivity.

There was no significant statistical correlation between HER 2 NEU over expression and invasiveness of the tumor.

It is inferred that 10% of invasive tumors showed strong membranous positivity whereas 87% of non muscle invasive tumor did not express HER 2 NEU.

CORRELATING THE EXPRESSION OF HER 2 NEU WITH GENDER:

In this study, of 52 cases, males constituted 69% (36 cases) and females accounted for 31% (16 cases). Out of 36 male patients, 31 patients (86%) had negative to weak membranous positivity, 3 patients (8%) expressed equivocal staining pattern and 2 patients (6%) expressed strong membranous positivity.

Of 16 female patients, 13 (81%) had negative to weak membranous positivity, 1 case (6%) expressed equivocal staining pattern and 2 cases (13%) expressed strong membranous positivity.

On considering the gender with immunohistochemical expression of HER 2 NEU, 6% males and 13% females expressed strong membranous positivity. There is not much correlation between gender and immunohistochemical expression of HER 2 NEU because the distribution of cases among males and females is not uniform and there is an inherent tendency that urothelial carcinoma is more common in males than in females [(2)].

CORRELATING THE EXPRESSION OF HER 2 NEU WITH AGE:

In this study, the patient's age has been divided into 5 groups starting from 30-40 years, 41-50, 51-60, 61-70 and above 70 years of age.

Among 52 cases, strong membranous positivity was observed in the following age group: 30-40 years (1 case), 51-60 years (1 case), 61-70 years (2 cases). It is evident that there is variable expression of HER 2 NEU among each age group and its expression is independent of this variable.

CORRELATING THE EXPRESSION OF HER 2 NEU WITH SIZE:

In this study, the size of the tumor is grouped into 3 ranges- 1.5-3cm, 3-5cm and more than 5cm. Of 52 urothelial carcinoma cases, strong membranous positivity was expressed in 2 ranges: 3-5 cm (3 case) and more than 5cm (1 case).

It is clear that there is variable expression of HER 2 NEU among each size range and its expression is independent of this variable.

CORRELATING THE EXPRESSION OF HER 2 NEU WITH THE STAGE OF TUMOR:

Of 23 stage I tumors, only one case (4%) expressed strong membranous positivity, 2 cases (9%) had equivocal staining pattern and 20 cases (87%) revealed negative staining pattern.

Among 25 stage II tumors, 20 cases (80%) expressed negative staining pattern, 2 cases 8%) had equivocal staining pattern and 3 cases (12%) showed strong membranous positivity.

Out of 4 stage III tumors, all (100%) 4 cases revealed negative staining pattern.

It is observed that immunohistochemical expression of HER 2 NEU is independent of the stage of tumor.

In the study conducted by Charfi et al, HER 2 NEU over expression was significantly correlated with the grade but not with the stage, invasiveness of the tumor and clinical variables like age, gender, tumor size and this correlated with the present study. Patients who over expressed HER 2 NEU protein can be considered for targeted therapy as in breast carcinoma.

In the present series, on comparing the immunohistochemical expression of HER 2 NEU with various clinical and pathological variables, it is evident that there is a significant statistical correlation between HER 2 NEU expression and grade of the tumor and its expression is not affected by invasiveness of the tumor, gender, age, tumor size and stage of the tumor.

IMMUNOHISTOCHEMICAL EXPRESSION OF P53:

The criteria of nuclear positivity in more than 10% of the tumor cells is considered as over expression of P53 protein and this criteria is strictly followed while interpreting the expression of P53 protein.

CORRELATING THE EXPRESSION OF P53 WITH GRADE:

Of 26 high grade urothelial carcinoma, 13 cases (50%) showed nuclear positivity in more than 10% of the tumor cells and considered to over express P53 protein, while remaining 13 cases (50%) expressed negative staining pattern. The 13 cases (50%) which expressed negative P53 staining did not fulfill the 10% criteria for over expression and P53 expression was seen in less than 10% of the tumor cells in most of these cases. Of 26 low grade urothelial carcinoma, all cases (100%) stained negative for P53 staining as the staining pattern did not meet the 10% criteria for over expression.

It is evident that 50% of high grade urothelial carcinoma over expressed P53 protein whereas 100% of low grade urothelial carcinoma did not over express P53 protein. It is clear that P53 over expression correlated with the grade of the tumor.

CORRELATING THE EXPRESSION OF P53 WITH INVASIVENESS OF THE TUMOR:

Of 29 muscle invasive tumors, 18 cases (62%) expressed negative P53 staining and 11cases (38%) over expressed P53 protein.

Among 23 non muscle invasive tumors, 21cases (91%) expressed negative P53 staining and 2 cases (9%) over expressed P53 staining.

It is inferred that 91% of non muscle invasive tumors were negative for P53 staining whereas 62% of muscle invasive urothelial carcinoma over expressed P53 protein. Over expression of P53 protein is associated with invasiveness of the tumor.

CORRELATING THE EXPRESSION OF P53 WITH GENDER:

Of 36 male patients, 29 males (81%) did not over express P53 & 7 males (19%) over expressed P53 protein.

Among 16 females, 10 patients (63%) showed negative P53 expression whereas 6 patients (37%) over expressed P53 protein. Thus P53 over expression is independent of gender.

CORRELATING THE EXPRESSION OF P53 WITH AGE:

As already mentioned the age of the patients is divided into 5 groups. Of 52 cases, 13 cases (25%) over expressed P53 protein, the maximum number of

cases fall in the age range of 61-70 years (4 cases) followed by 51-60 age range (3 cases). It is evident that P53 expression is independent of age.

CORRELATING THE EXPRESSION OF P53 WITH SIZE OF THE TUMOR:

Of 52cases, 13 cases (25%) which over expressed P53 protein had variable range of tumor size. Among 13 cases which over expressed P53 protein, the maximum number of cases (8 cases) fall in the size range of 3-5cm followed by 3 cases which falls in the range more than 5cm. Because of this variable staining pattern, the P53 over expression does not correlate with the size of the tumor.

CORRELATING THE EXPRESSION OF P53 WITH STAGE:

Among 23 stage I tumors, 21 cases (91%) showed negative staining pattern for P53 and 2 cases (9%) over expressed P53 protein. Of stage II tumors, 17 cases (68%) showed negative P53 staining and 8 cases (32%) over expressed P53 protein. Out of 4 stage III tumors, 1 case (25%) showed negative P53 staining and 3 cases (75%) over expressed P53 protein.

In this study, 9% of stage I, 32% of stage II and 75% of stage III tumors over expressed P53 protein and it is evident that over expression of P53 protein is associated with higher stage of the tumor.

In urothelial carcinoma, P53 over expression was reported in 30.8% to 60% of high grade tumors [157,158]. According to Charfi et al, P53 was over expressed in 31.8% of tumors and correlated with the grade and the stage of the

tumor. In the present series, P53 over expression correlated with the grade, stage and invasiveness of the tumor. Several variables like gender, age, tumor size does not affect the expression of P53.

A prognostic significance was noted for P53 staining in both univariate and multivariate analysis[159]. According to Shariat et al, P53 is the strongest predictive marker in determining the outcome of the patient.

IMMUNOHISTOCHEMICAL EXPRESSION OF P63:

The criteria of less than 90% of the tumor cells exhibiting loss of nuclear positivity is considered as decreased expression of P63. In this study, 26 high grade and 26 low grade urothelial carcinoma is included. Of 26 high grade urothelial carcinoma, 22 cases (85%) showed decreased expression of P63 by fulfilling the criteria for decreased expression and remaining 4 cases (15%) retained their normal P63 staining. These 4 cases showed more than 90% nuclear positivity and hence considered as normal P63 expression.

Among 26 low grade urothelial carcinoma, 4 cases (15%) showed decreased P63 expression whereas 22 cases (85%) retained 90% nuclear positivity for P63 protein. It is evident that 85% of high grade urothelial carcinoma showed decreased P63 expression, while 85% of low grade urothelial carcinoma retained their 90% nuclear positivity and considered as normal expression of P63 protein.

It is observed that most high grade urothelial carcinoma showed decreased expression of P63 protein while low grade urothelial carcinoma retained their

normal P63 expression. Thus decreased expression of P63 protein correlated with the grade of tumor.

CORRELATING THE EXPRESSION OF P63 WITH THE INVASIVENESS OF THE TUMOR:

Of 29 muscle invasive tumors, 23 cases(79%) showed decreased expression of P63, while 6 cases (21%) retained their normal expression. Among 23 non muscle invasive tumors, 3 cases (13%) showed decreased expression of P63 and remaining 20 cases (87%) retained their normal P63 staining.

It is evident that 79% of muscle invasive tumors showed decreased expression of P63 while 87% of non muscle invasive tumors retained normal P63 expression. Thus decreased expression of P63 correlates with the invasiveness of the tumor.

CORRELATING THE EXPRESSION OF P63 WITH GENDER:

Of 36 male patients, 18 cases (50%) showed decreased expression of P63 and 18 cases (50%) retained normal expression of P63. Among 16 females, 8 cases (50%) retained normal expression and remaining 8 (50%) showed decreased expression of P63. Thus, 50% males and 50% females showed decreased expression of P63 protein. It is evident that decreased expression of P63 is independent of gender and is not statistically significant.

CORRELATING THE EXPRESSION OF P63 WITH AGE:

Of 52 cases, 26 cases (50%) showed decreased expression of P63, remaining 26 cases (50%) retained normal expression of P63 protein. Among 26 cases which showed decreased expression of P63, the maximum number of casesfall in the age range of 61-70years (11 cases), followed by 51-60 years age range (8 cases). It is evident that decreased expression of P63 is independent of age due to variable staining pattern that is observed in each age range.

CORRELATING THE EXPRESSION OF P63 WITH SIZE:

Of 52 cases, 26 cases (50%) which showed decreased expression of P63 protein had variable range of tumor size. Among 26 cases which showed decreased expression of P63 protein, the maximum number of cases (15 cases) fall in the size range of 3-5cm followed by 9 cases which falls in the range more than 5cm size. There was statistical correlation between decreased expression of P63 protein and tumor size.

CORRELATING THE EXPRESSION OF P63 WITH STAGE:

Among 23 stage I tumors, 3 cases (13%) showed decreased expression of P63 and 20 cases (87%) retained normal expression of P63 protein.

Of 25 stage II tumors, 19 cases (76%) showed decreased expression of P63 and 6 cases (24%) retained normal expression of P63.

Of 4 stage III tumors, all 4 cases (100%) showed decreased expression of P63 protein.

In this study, 13% of stage I, 76% of stage II and 100% of stage III tumors showed decreased expression of P63. A significant statistical correlation was present between decreased expression of P63 and stage of the tumor. It is evident that as stage advances, the decreased expression of P63 protein increases and can influence the outcome of the patient.

According to Charfi et al, decreased expression of P63 was observed in 31.8% of high grade urothelial tumors and decreased expression of P63 correlated with the tumor grade and stage [160]. In the present study also decreased expression of P63 correlates with the tumor size, grade, invasiveness and the stage. P63 expression does not depend on the clinical variables like age and gender.

Several studies reported that decreased expression of P63 is associated with advanced disease and poorer prognosis [161].

CORRELATING HER 2 NEU EXPRESSION WITH P53 EXPRESSION:

Of 52 cases, 13 cases (25%) over expressed P53 and 39 cases (75%) showed negative staining for P53 protein. Among 13 cases which over expressed P53, 9 cases (69%) showed negative to weak membranous staining for HER 2 NEU and 4 cases (31%) showed strong membranous positivity .

Out of 39 cases which showed negative staining for P53 protein, 35 cases (90%) showed negative to weak membranous positivity and 4 cases (10%) showed equivocal staining pattern for HER 2 NEU. P value was significant and

statistical correlation was observed between HER 2 NEU expression and P53 expression.

CORRELATING THE EXPRESSION OF HER 2 NEU WITH P63 EXPRESSION:

Of 52 cases, 26 cases (50%) showed decreased expression of P63 protein and remaining 26 cases (50%) retained normal expression of P63 protein. Among 26 cases which showed decreased expression of P63 protein, 21 cases (81%) showed negative to weak membranous positivity for HER 2 NEU, 1 case (4%) had equivocal staining pattern and 4 cases (15%) showed strong membranous positivity for HER 2 NEU.

The remaining 26 cases (50%) which retained normal expression of P63 protein, 23 cases (88%) showed negative to weak membranous positivity for P63 protein and 3 cases (12%) showed equivocal staining pattern for HER 2 NEU.

There was a significant statistical correlation between HER 2 NEU over expression and P63 expression.

CORRELATING THE EXPRESSION OF P53 WITH P63:

Of 52 cases, 26 cases (50%) showed decreased expression of P63 protein and 26 cases (50%) retained normal expression of P63. Among 26 cases which showed decreased expression of P63, 15 cases (58%) showed negative P53 staining and 11 cases (42%) over expressed P53 protein.

Of 26 cases which retained normal expression of P63 protein, only 2 cases (8%) over expressed P53 protein and remaining 24 cases (92%) showed negative staining for P53 protein.

A significant correlation was observed between over expression of P53 protein and decreased expression of P63 protein.

In the study performed by Charfi et al, HER 2 NEU over expression was correlated with decreased expression of P63 but not with P53 over expression. In the present study, HER 2 expression correlated with P53 over expression and decreased expression of P63. Similarly, decreased expression of P63 correlated with P53 over expression.

Summary

SUMMARY

- The incidence of urothelial carcinoma in our Institute was 68%.
- The incidence of high grade carcinoma was 55% and that of low grade urothelial carcinoma was 45%.
- Most high grade urothelial carcinomas were muscle invasive (85%) and most low grade carcinomas were non muscle invasive (76%).
- The peak incidence of urothelial carcinoma was above 50 years of age and advanced age was associated with higher grade and stage of the tumor.
- Males constituted 69% and females accounted for 31% of cases.
- The most common site of urothelial carcinoma was lateral wall followed by posterolateral wall.
- The mean size of the tumor in this study was 3-5cm.
- A significant statistical correlation was observed between size and invasiveness and no correlation was observed between size and grade & stage.
- The sensitivity of urine cytology was higher in high grade urothelial carcinoma than in low grade urothelial carcinoma.
- Most high grade carcinomas were stage II & III and low grade urothelial carcinomas were stage I & II.
- A significant statistical correlation was observed between grade and the stage of the tumor.

- Immunohistochemical expression of HER 2 NEU showed a significant statistical correlation with tumor grade and no such correlation was observed with invasiveness, stage, age, gender and size of the tumor.
- Immunohistochemical expression of P53 showed correlation with tumor grade, stage, invasiveness and no correlation with age, gender and tumor size.
- Immunohistochemical expression of P63 showed correlation with grade, invasiveness, stage, size and no correlation with age, gender.
- A significant statistical correlation was observed between HER 2 NEU expression, P53 expression and P63 expression.
- There was a correlation between P53 expression and P63 expression.

Conclusion

CONCLUSION

This is a hospital based study and may not represent the true incidence of the disease in the community. The majority of patients are over 50 years of age and there is an overwhelming male preponderance of urothelial carcinoma. As many patients presented with advanced stage disease, the need for palliative treatment is increasing and because of the advanced age, the mortality associated with surgery also increases. This study helped in evaluating the new molecular markers involved in the pathogenesis of urothelial carcinoma. The immunohistochemical expression of HER 2 NEU, P53 and P63 is evaluated and correlated with various clinico pathological variables.

HER 2 NEU over expressing patients can be considered for targeted therapy and over expression is associated with poorer prognosis. Similarly decreased expression of P63 and over expression of P53 is associated with advanced stage and poorer outcome.

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Annexures

ANNEXURE- I

WHO CLASSIFICATION OF TUMORS OF THE UROTHELIAL TRACT-2016

Urothelial tumors

Infiltrating urothelial carcinoma

Nested, including large nested
Microcystic
Micropapillary
Lymphoepithelioma-like
Plasmacytoid/signet ring/diffuse
Sarcomatoid
Giant cell
Poorly differentiated
Lipid-rich
Clear cell

Non invasive urothelial neoplasms

Urothelial carcinoma in situ
Non-invasive papillary urothelial carcinoma,low grade
Non-invasive papillary urothelial carcinoma,high-grade
Papillary urothelial neoplasm of low malignant potential
Urothelial papilloma
Inverted urothelial papilloma
Urothelial proliferation of uncertain malignant potential
Urothelial dysplasia

Squamous cell neoplasms

Pure squamous cell carcinoma
Verrucous carcinoma
Squamous cell papilloma

Glandular neoplasms

Adenocarcinoma,NOS
  Enteric
  Mucinous
  Mixed
Villous adenoma

Urachal carcinoma

Tumors of Mullerian type

Clear cell carcinoma
Endometrioid carcinoma

Neuroendocrine tumors

Small cell neuroendocrine carcinoma
Large cell neuroendocrine carcinoma
Well differentiated neuroendocrine tumor
Paraganglioma

Melanocytic tumors

Malignant melanoma
Naevus
Melanosis

Mesenchymal tumors

Rhabdomyosarcoma
Leiomyosarcoma
Angiosarcoma
Inflammatory myofibroblastic tumor
Perivascular epithelioid cell tumor-benign,malignant
Solitary fibrous tumor
Leiomyoma
Haemangioma
Granular cell tumor
Neurofibroma

Urothelial tract haematopoietic and lymphoid tumors

Miscellaneous tumors

Carcinoma of Skene, Cowper and Littre glands
Metastatic tumors and tumors extending from other organs
Epithelial tumors of the upper urinary tract
Tumors arising in a bladder diverticulum
Urothelial tumors of the urethra

ANNEXURE II

TNM Staging for Bladder Carcinoma

Primary Tumor (T)

TX -Primary tumor cannot be assessed

TO -No evidence of primary tumor

Ta -Noninvasive papillary carcinoma

Tis -Carcinoma in situ

T1 -Tumor invades subepithelial connective tissue

T2 -Tumor invades muscle

pT2a -Tumor invades superficial muscle

pT2b -Tumor invades deep muscle

T3 -Tumor invades perivesical tissue

pT3a -Microscopically

pT3b -Macroscopically (extravesical mass)

T4 -Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, or abdominal wall

T4a -Tumor invades the prostatic stroma, uterus, vagina

T4b -Tumor invades the pelvic wall, abdominal wall

Regional Lymph nodes (n)

NX -Regional lymph nodes cannot be assessed

N0 -No regional lymph node metastasis

N1 -Single lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac or presacral lymph node)

N2 -Multiple regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac or presacral lymph node)

N3 -Lymph node metastasis to the common iliac lymph nodes

Distant metastasis (M)

M0 -No distant metastasis (no pathologic M0; use clinical M to complete stage group)

M1 -Distant metastasis

AJCC pathologic stage groups

Stage Oa	Ta	N0	M0
Stage Ois	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2a	N0	M0
	T2b	N0	M0
Stage III	T3a	N0	M0
	T3b	N0	M0
	T4a	N0	M0
Stage IV	T4b	N0	M0
	Any T	N1	M0
	Any T	N2	M0
	Any T	N3	M0
	Any T	Any N	M1

ANNEXURE III

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4 micron thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to positively charged slides.
2. The slides were incubated at 58^{o}C for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in buffer for 5 minutes x 2 changes.
13. Cover the sections with protein block for 15 minutes.
14. The sections were drained without washing and appropriate antibody was applied over the sections and incubated for one hour.
15. The slides were washed in wash buffer for 5 minutes x 2changes.
16. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml of DAB buffer.
17. DAB substrate solution was applied on the sections for 2 minutes.
18. The slides were washed in distilled water for 5 minutes.
19. The sections were counterstained with Hematoxylin stain.
20. The slides were washed in running tap water for 3 minutes.
21. The slides were air dried and mounted with DPX.

Master chart

MASTER CHART

S. NO	BX NO	AGE	SEX	CLINICAL DIAGNOSIS	PROCEDURE DONE	CYSTOSCOPY	IMAGING	SITE OF GROWTH	URINE CYTOLOGY	GRADE	INVASIVE NESS	STAGE	HER 2 NEU	P53	P63
1	88/17	45	M	CA BLADDER	TURBT	papillary growth	GROWTH IN LATERAL WALL 2x1.5 CM	LATERAL WALL	DESCRIPTIVE	HIGH	NMI	I	1+	OVER EXPRESSED	NORMAL
2	254/17	55	M	CA BLADDER	PALLIATIVE CYSTECTOMY	papillary growth	RECURRENT MASS 4X3 CM	LATERAL WALL	NO ATYPICAL CELLS	HIGH	MI	II	2+	NEGATIVE	DECREASED
3	260/17	65	M	CA BLADDER	TURBT	papillary growth	5X4 CM PAPILLARY GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	1+	NEGATIVE	NORMAL
4	318/17	70	M	CA BLADDER	TURBT	papillary growth	MULTIPLE FOCAL BLADDER MALIGNANCY LARGEST MEASURING 5X3CM	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	3+	OVER EXPRESSED	DECREASED
5	447/17	60	M	CA BLADDER	TURBT	papillary growth	GROWTH IN LATERAL WALL 2X2CM	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	2+	NEGATIVE	NORMAL
6	1173/17	57	F	CA BLADDER	TURBT	papillary growth	4X4 CM GROWTH	LATERAL WALL	ACELLULAR	LOW	NMI	I	0	NEGATIVE	NORMAL
7	1228/17	55	M	RECURRENT CA BLADDER	TURBT	papillary growth	3X3CM GROWTH	POSTERIOR WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
8	1265/17	70	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	5X4CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
9	1589/17	60	F	CA BLADDER	TURBT	papillary growth	4X3CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	3+	OVER EXPRESSED	DECREASED
10	1774/17	60	M	CA BLADDER	TURBT	papillary growth	2X1CM GROWTH	LATERAL WALL	NO ATYPICAL CELLS	LOW	NMI	I	0	NEGATIVE	NORMAL
11	1923/17	56	F	CA BLADDER	ANTERIOR PELVIC EXENTERATION	papillary growth	1.7X1.4CM GROWTH	POSTEROLATERAL WALL	DESCRIPTIVE	LOW	NMI	I	0	NEGATIVE	NORMAL
12	2101/17	70	F	CA BLADDER	TURBT	papillary growth	1.5X1CM GROWTH	LATERAL WALL,BASE	DESCRIPTIVE	LOW	NMI	I	2+	NEGATIVE	NORMAL
13	2474/17	70	M	CA BLADDER	TURBT	papillary growth	6X3CM BROAD BASED FIRM MASS	TRIGONE	NO ATYPICAL CELLS	LOW	NMI	I	1+	NEGATIVE	DECREASED
14	2476/17	34	M	CA BLADDER	TURBT	papillary growth	2X1.5CM GROWTH	LATERAL WALL	ACELLULAR	LOW	NMI	I	1+	NEGATIVE	NORMAL
15	2572/17	46	M	CA BLADDER	TURBT	papillary growth	5.6X7 CM IRREGULAR ENHANCING LESION	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	MI	II	0	NEGATIVE	NORMAL
16	2904/17	53	M	CA BLADDER	TURBT	papillary growth	2X1 CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	1+	NEGATIVE	NORMAL
17	3097/17	40	M	CA BLADDER	TURBT	papillary growth	7X4CM GROWTH	ANTERIOR WALL	POSITIVE FOR MALIGNANCY	HIGH	NMI	I	3+	OVER EXPRESSED	DECREASED
18	3196/17	60	M	CA BLADDER	TURBT	papillary growth	3X2CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED
19	3347/17	55	M	CA BLADDER	TURBT	papillary growth	7X3CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED
20	3362/17	66	F	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	7X7CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	III	1+	NEGATIVE	DECREASED
21	3401/17	70	M	CA BLADDER	TURBT	papillary growth	4X3CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	OVER EXPRESSED	DECREASED
22	3558/17	75	M	CA BLADDER	TURBT	papillary growth	8X6CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	1+	NEGATIVE	DECREASED
23	3559/17	68	F	CA BLADDER	TURBT	papillary growth	5X4CM GROWTH	ANTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	3+	OVER EXPRESSED	DECREASED
24	3696/17	73	M	CA BLADDER	TURBT	papillary growth	2.5X2.5CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
25	3850/17	35	F	CA BLADDER	TURBT	papillary growth	2X2CM GROWTH	LATERAL WALL	NO ATYPICAL CELLS	HIGH	MI	II	1+	NEGATIVE	DECREASED
26	4274/17	66	M	CA BLADDER	TURBT	papillary growth	4X4 CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	1+	NEGATIVE	DECREASED
27	4320/17	64	M	CA BLADDER	TURBT	papillary growth	1.5X1CM GROWTH	LATERALWALL	NO ATYPICAL CELLS	LOW	NMI	I	1+	NEGATIVE	NORMAL

28	1204/16	44	F	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	5X5CM GROWTH	POSTEROLATERAL WALL	ACUTE INFLAMMATORY PATHOLOGY	HIGH	MI	III	0	OVER EXPRESSED	DECREASED
29	1302/16	65	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	4X3CM GROWTH	LATERAL WALL,BASE	POSITIVE FOR MALIGNANCY	HIGH	MI	III	0	OVER EXPRESSED	DECREASED
30	1577/16	78	M	CA BLADDER	TURBT	papillary growth	3.8X2.6CM GROWTH	LATERAL WALL	SUSPICIOUS OF MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
31	2297/16	47	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	2X1CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
32	2438/16	58	M	CA BLADDER	TURBT	papillary growth	3X2CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED
33	2561/16	84	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	MULTIPLE ECHOIC LESION 1.5X1.5CM	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	OVER EXPRESSED	NORMAL
34	4779/16	60	M	CA BLADDER	TURBT	papillary growth	9X5CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	1+	NEGATIVE	NORMAL
35	4848/16	57	F	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	6X5CM GROWTH	LATERAL WALL	SUGGESTIVE OF MALIGNANCY	LOW	MI	II	0	NEGATIVE	NORMAL
36	5017/16	36	M	CA BLADDER	TURBT	papillary growth	3X2CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED
37	5136/16	65	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	6X5CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	MI	II	0	NEGATIVE	NORMAL
38	7897/16	80	F	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	7X5CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	III	0	OVER EXPRESSED	DECREASED
39	7983/16	55	F	CA BLADDER	TURBT	papillary growth	4X3CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
40	8028/16	75	M	CA BLADDER	TURBT	papillary growth	3X2CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	OVER EXPRESSED	DECREASED
41	8091/16	65	M	CA BLADDER	TURBT	papillary growth	1.5X1.5CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	DECREASED
42	8476/16	70	F	CA BLADDER	TURBT	papillary growth	6X5CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	NORMAL
43	8570/16	75	F	CA BLADDER	TURBT	papillary growth	2X1CM GROWTH	POSTERIOR WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
44	8615/16	53	F	CA BLADDER	TURBT	papillary growth	5X3CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	OVER EXPRESSED	DECREASED
45	8798/16	67	M	CA BLADDER	TURBT	papillary growth	4X4CM GROWTH	POSTEROLATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED
46	9173/16	65	M	CA BLADDER	TURBT	papillary growth	3X2CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	NMI	I	0	NEGATIVE	NORMAL
47	9283/16	55	F	CA BLADDER	TURBT	papillary growth	5X3CM GROWTH	LATERAL WALL	ACUTE INFLAMMATORY PATHOLOGY	HIGH	MI	II	0	OVER EXPRESSED	DECREASED
48	9323/16	55	F	CA BLADDER	TURBT	papillary growth	2X1CM GROWTH	LATERAL WALL	NO ATYPICAL CELLS	LOW	NMI	I	1+	NEGATIVE	NORMAL
49	9580/16	68	M	CA BLADDER	RADICAL CYSTECTOMY	papillary growth	5X4CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	MI	II	0	NEGATIVE	DECREASED
50	11409/16	55	M	CA BLADDER	PALLIATIVE CYSTECTOMY	papillary growth	9X8CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	LOW	MI	II	1+	NEGATIVE	DECREASED
51	11588/16	55	M	CA BLADDER	TURBT	papillary growth	7X5CM GROWTH	POSTEROLATERAL WALL	ACUTE INFLAMMATORY PATHOLOGY	HIGH	MI	II	2+	NEGATIVE	NORMAL
52	11985/16	64	M	CA BLADDER	TURBT	papillary growth	6X4CM GROWTH	LATERAL WALL	POSITIVE FOR MALIGNANCY	HIGH	MI	II	0	NEGATIVE	DECREASED